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PHENOTYPIC AND MOLECULAR CHARACTERISTICS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM STORED PATIENT SAMPLES IN MISURATA HOSPITALS AND POULTRY FROM COMMERCIAL MARKETS, LIBYA

by

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15 May 2019

DECLARATION

I declare that "**Phenotypic and Molecular Characteristics of Methicillin-Resistant *Staphylococcus aureus* Isolates from Stored Patient Samples in Misurata Hospitals and Poultry from Commercial Markets, Libya**," is my own work, that it has not been submitted for any other degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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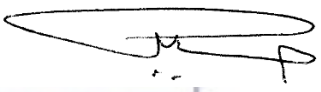
Student Number: 3280456 **:** 

Date Signed **:** 15 May 2019

DEDICATION

I dedicate this thesis to my:

- Father, Ali Mohamed Elakrout, and mother, Aisha Milad Gibranr, and my loving wife, Aisha Ibrahim Farhat.
- My brothers, Mohamed Ali Elakrout and Snoussi Ali Elakrout.
- My sisters, Rogia Ali Elakrout and Halima Ali Elakrout.
- My nephews, Ali Mohamed Elakrout, Hamza Mohamed Elakrout, Haltham Mohamed Elakrout and Ibrahim Mohamed Elakrout.
- My nieces, Aisha Mohamed Elakrout, Zahor Mohamed Elakrout, Salthania Mohamed Ala- jaley and Salthania Mohamed Alajaley.
- My extended family and friends, Ibrahim Mohamed Farhat (Alfetey), Salema abdullah Alsnoussi, Soiad Alhadi Alkrazab and Hamedia Abduikhalig.

LITERARY QUOTATIONS

In the name of Allah, the most gracious most merciful.

Allah will raise those who have believed among you and those who were given knowledge, by degrees. And Allah is Acquainted with what you do.

—Quran surah Al-Mujadilah 4 (QS 58: 4)

"There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring 'fastness' [resistance]."

—Alexander Fleming, 1946

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ABSTRACT

Background

The emergence of virulent and drug-resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) is a global public health burden. The World Health Organization (WHO) has placed MRSA and vancomycin-intermediate-sensitive *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) on a high global priority pathogens list of antibiotic-resistant bacteria to promote the research and development of novel and effective antibiotic therapeutic rationales. Uncomplicated *S. aureus* bacteraemia (e.g., mild skin infections) may be treatable with the conventional regimens of antibiotics, but resistance strains of the bacteria (e.g., invasive infections), often persist as a high load of bacterial DNA in blood, and has been linked to increased mortality in world populations, irrespective of country or location. Several lines of evidence imply that combinations of vancomycin (a glycopeptide antibiotic that targets cell wall synthesis) and ß-lactam antibiotics that target the penicillin-binding proteins (PBPs) improve clearance of MRSA bloodstream infections (BSIs).

However, acquired resistance to virtually the entire spectrum of ß-lactams hinders the therapeutic benefit of such antibiotic combinations. Hospital/health care-associated resistant *S. aureus* infections (HA-MRSA) and community-associated resistant *S. aureus* infections (CA-MRSA), continue to be among the most common and challenging life-threatening infections worldwide. Furthermore, the increased rate of resistance among MRSA and vancomycin-resistant *Enterococcus* (VRE) underscore the dire need for the discovery of novel anti-MRSA and anti-VRE compounds. The degree of ß-lactam resistance varies among clinical MRSA isolates, particularly with regard to those mediated by chromosomal mutations and the novel exogenous resistance gene which encodes PBP2a, i.e., *mecA*. PBP2a is the key resistance factor of ß-lactams, but the evolution of this *mecA* gene product and its mechanisms remain elusive.

Nonetheless, it is widely accepted that in MRSA, PBP2a reduces the binding affinity to ß-lactam antibiotics, rendering them ineffective. Besides HA-MRSA and CA-MRSA, colonization by livestock-associated MRSA (LA-MRSA) presents a major threat to both animal and human health, e.g., MRSA clonal complex (CC) 398 has spread from pigs to humans, but rarely from person to person. Even though LA-MRSA CC398 has been deemed less virulent than other MRSA strains, it particularly colonizes pig farmers. Recent studies indicate that an increasing number of people are being infected with LA-MRSA CC398. LA-MRSA in mink is considered a human health hazard to farmers and farm workers, who handle the animals and are at risk of bites and scratches from colonized sites. Likewise, MRSA is also present in rabbits, cattle (e.g., dairy cows) and poultry, and, as such, has become an emerging threat to public health because of the spread from animals to humans via animal husbandry, and the health care and food processing industry.

Generally, the irrational use of antibiotics is the main cause of the emergence of antibiotic resistant *S. aureus* strains. Globally, in both developed and developing countries, MRSA represents a serious public health concern because of the rapid spread of this bacterium around the world coupled with the evolution of new genetically distinct HA-MRSA, CA-MRSA and LA-MRSA strains. Additionally, the development of cross-resistance to other non-ß-lactams, including vancomycin, has only exacerbated the burden of MRSA infections. In many countries such as the USA, UK, Europe and Iran, the MRSA epidemic has been well-documented. However, in South America and Africa, there seems to be a paucity on available data concerning MRSA strains. Equally, in Libya, detailed information is lacking regarding MRSA contagiousness risk, but conscious efforts are currently being directed at understanding the molecular epidemiology of *S. aureus* isolates, not only in terms of the persistence and spread of CA-MRSA, HA-MRSA and LA-MRSA, but also to focus awareness on prudent antibiotic prescription policy and use, as well as prevention and control of MRSA transmission in hospital, community and food production settings.

The aim of this study was to compare antibiotic sensitivities of *Staphylococcus aureus* isolates in human patient samples from Misurata hospitals and laboratories as well as poultry samples from commercial markets in Libya. The objectives of the study were to (1) analyze laboratory and hospital samples from patients as well as poultry samples for bacterial and fungal growth, using standard bacterial isolate identification and antibiotic susceptibility tests; (2) compare traditional bacterial culture methods that are used to measure MRSA strains with modern molecular methods to isolate the *mecA1* and *mecA2* genes, using PCR; (3) determine the diagnostic profile of the bacterial and fungal species in patient and poultry specimens; (4) evaluate the *Staphylococcus aureus* antibiotic sensitivity and resistance profiles for patient and poultry samples; (5) compare *Staphylococcus aureus* antibiotic sensitivity and resistance profiles in patient specimens according to gender, age group and location (site collected); (6) compare *Staphylococcus aureus* antibiotic sensitivity and resistance profiles in poultry samples according to location and different parts of the chicken after slaughter; (7) identify and detect the MRSA contamination in chicken samples.

Methods

In this study, 657 laboratory and hospital samples from patients were collected. Patient samples were collected from blood culture, cerebrospinal fluid (CSF), eye swab, lung swab, semen, sputum, stool, swabs, swabs from operation, throat swabs, urine and wound swabs. The samples were taken at the intensive care units (ICUs), laboratories and surgical departments of the three main hospitals, as well as at a private clinical laboratory, all located in Misurata, Libya. These institutions are the National Cancer Institute Misurata (NCIM), the Central Hospital Misurata (CHM) the laboratory of the Al Saffwa International Hospital (AIH), and the Misurata Central Laboratory (MSL). In addition, 361 swabs from chicken samples were collected from five locations of which 72 showed no bacterial growth, while 289 showed bacterial growth. The locations were Abdurrahman Abahy, Alfetory, Baser, Isolate and Alkherobp Market. Strains of *Staphylococcus aureus* were collected in one year between 1

January 2014 and 31 December 2014. Samples from human and poultry were collected. Antimicrobial susceptibility testing was performed by the disc diffusion method for selected antibiotics. The following methods were used for *S. aureus* identification and antibiotic susceptibility testing: the blood agar test, Gram-stain test, mannitol salt agar test, the catalase test, the coagulase test, the Mueller-Hinton agar test, minimum inhibitory concentration (MIC) tests and the polymerase chain reaction (PCR). MRSA was determined by the disc diffusion method applied to oxacillin.

Results

Of the total number of 657 patient samples, 449 (68%) were collected from hospital departments (ICU, Inpatients, Newborns and Outpatients) and 208 (32%) were collected from a laboratory. The majority of the samples (n=378; 58%) were collected from Central Hospital Misurata (CHM), followed by the National Cancer Institute of Misurata (NCIM, n=128; 19%), Assafwa International Hospital (AIH, n=110; 17%) and Misurata Central Laboratory (MCL, n=41; 6%). In terms of gender distribution, the number of specimens collected from females (n=256; 58%) exceeded that of males (n=186; 42%), i.e., of a total of 442 specimens. The highest number of specimens derived from the 30–49-year old group (n=151; 39%).

The majority of specimens collected and tested were in the following order: urine (37.4%), swabs (31.5%), semen (17%) and cerebrospinal fluid (CSF) (9.2%), swabs from operations (2.4%). The remainder of samples, i.e., blood culture, eye swab, lung swab, sputum, stool, throat swab, and wound swab each constituted less than 1% of the total number of specimens collected and tested. The number of positive samples of the Gram-stain test was 195 (55%), while the number of negative samples of the Gram-stain test was 161 (45%). The positive samples of the catalase test numbered 301 (93%), while the number of catalase-negative samples was 21 (7%). Furthermore, the number of the positive samples of the coagulase test was 84 (49%), and the number of coagulase-negative samples was 87 (51%). The growth of bacteria on Mueller-Hinton agar was found to be 100% *S. aureus*.

A total of 37 MRSA from 73 strains of *S. aureus* were identified. The highest sensitivities of MRSA were towards vancomycin (96%), amikacin (89%), streptomycin (82%) and gentamicin (81%), while lower sensitivities were noted for erythromycin (25%), oxacillin (49%), kanamycin (49%) and tetracycline (51%). The highest rate of oxacillin-resistant *S. aureus* (OXRSA) was observed in males (61.5%) whereas the observed OXRSA for females was 44.1%. The highest rate for oxacillin-sensitive *S. aureus* (OXSSA) was 55.9% for females and 38.5% in males. Overall, the rate for OXRSA (51.7%) exceeded that of OXSSA (48.3%). Males had the highest vancomycin-resistant *S. aureus* (VRSA) rate (3.8%), whereas no VRSA was detectable in female samples. However, the highest vancomycin-sensitive *S. aureus* (VSSA) rate was observed in female samples (100%), while the VSSA rate in males was equally high at 96%. Overall, the VSSA rate was 98.3% compared to the VRSA rate of 1.7%, implying a high *S. aureus* sensitivity to vancomycin.

Levels of erythromycin intermediate-sensitive *S. aureus* (EISSA) in both female and male specimens were very low or negligible, i.e., 2.9% and 0%, respectively. Both female and male specimens exhibited high rates of erythromycin-resistant *S. aureus* (ERSA), i.e., 76.5% and 76.9%, respectively. The erythromycin-sensitive *S. aureus* (ESSA) profiles for females and males were 20.6% and 23.1%, respectively. Overall, ERSA (76.7%) exceeded ESSA (21.6%), while the EISSA was 1.7%. Detection of gentamicin-intermediate-sensitive *S. aureus* (GISSA) was very low in both females (5.9%) and males (3.8%) and thus relatively low overall (5%). Similarly, the detection of gentamicin-resistant *S. aureus* (GRSA) in both female (14.7%) and male (7.7%) specimens was relatively low, and the overall rate of GRSA was 11.7%. The highest rate for gentamicin-sensitive *S. aureus* (GSSA) in female specimens was 79.4% and in males it was 88.5%, and overall it was 83.3%, indicating a high level of bacterial sensitivity towards the antibiotic.

The highest kanamycin-resistant *S. aureus* (KRSA) rate was observed for females (58.8%) and for males it was 38.5%. By contrast, the kanamycin-sensitive *S. aureus* (KRSA) rate was

higher in males (61.5%) than in females (41.2%). Overall, both the KRSA and KSSA rates were 50.0%. The streptomycin-resistant *S. aureus* (SRSA) rates were 23.1% and 14.7% for males and females, respectively. Streptomycin-sensitive *S. aureus* (SSSA) profiles for females and males were very high, i.e., 85.3% and 76.9%, respectively. Overall, the SSSA rate (81.7%) exceeded the SRSA rate (18.3%), implying moderate levels of resistance to the antibiotic. In female specimens, no amikacin-intermediate-sensitive *S. aureus* (AISSA) was detected, whereas in males the AISSA rate was 3.8% compared to an overall AISSA rate of 1.6%. In both females and males, the amikacin-sensitive *S. aureus* (ASSA) rates were substantially high, i.e., 85.3% and 88.5%, respectively. The overall ASSA rate was also very high, i.e., 86.7%. Notwithstanding the encouraging high ASSA rates, the amikacin-resistant *S. aureus* (ARSA) rates for females and males, i.e., 14.7% and 7.7%, respectively, as well as an overall rate of 11.7% may become a health burden.

The rates of PCR positive (PCR+) and PCR negative (PCR-) expression of the *mecA1* and *mecA2* genes were verified in the different specimens. The highest PCR+ rate was observed in male specimens, i.e., 100%. In female specimens, the PCR+ rate was 93.4% and the PCR- rate was 6.7%. Overall the PCR+ rate was 96.7%. The rate for OXRSA in hospital samples was greater (51.7%) than that for laboratory samples (46.2%). The highest rate for OXSSA was noted for laboratory samples (53.8%) compared with 48.3% for hospital samples. Overall, i.e., for both hospital and laboratory, the rates for OXRSA (50.7%) and OXSSA (49.3%) were equivalent. The rate of VRSA was 15.4% in laboratory specimens vs 1.7% in hospital specimens. VSSA detection was high in both laboratory (84.6%) and hospital (98.3%) specimens. The proportions of tetracycline-resistant *Staphylococcus aureus* (TRSA) were almost similar in laboratory specimens (46.2%) and hospital specimens (50%), as were the proportions of tetracycline-sensitive *Staphylococcus aureus* (TSSA) in laboratory (53.8%) and hospital (50%) specimens. Thus, the overall rates of TRSA and TSSA in both laboratory and hospital samples were evenly matched, i.e., 49.3% and 50.7%, respectively. None of the laboratory samples showed any evidence of EISSA, but 1.6% of hospital samples did. ERSA

detection was high in both laboratory (61.5%) and hospital (76.7%) samples, but ESSA was lower in the aforementioned samples, i.e., 38.5% and 21.7%, respectively. GISSA rates in hospital samples was 5.0%, whereas no GISSA was detected in laboratory samples. The GRSA rate was higher in laboratory samples (30.8%) compared to hospital samples (11.7%). However, the detection of GSSA strains was greater in hospital samples (83.3%) compared to laboratory samples (69.2%). Overall, the GSSA rate (80.8%) was greater than the GRSA rate (15.1%), whereas the GISSA rate was relatively low (4.1%). The rates of KRSA in laboratory and hospital specimens were at similar levels, i.e., 53.8% and 50%, respectively. An almost similar trend was observed with KSSA, i.e., 46.2% and 50%, respectively. Overall, KRSA and KSSA levels were also equivalent, i.e., 50.7% and 49.3%, respectively. The detection levels for SSSA in laboratory and hospital samples were equivalent, i.e., 84.6% vs 81.7%. Similarly, SRSA in laboratory and hospital samples were almost identical, i.e., 15.4% vs 18.3%. Overall, the SSSA rate (82.2%) exceeded the SRSA (17.8%).

AISSA and ARSA strains were non-detectable in laboratory specimens, whereas ASSA were observed in all laboratory specimens (100%). Likewise, ASSA strains were detected in the majority of hospital samples (86.7%), ARSA to a much lesser extent (11.7%), and AISSA marginally (1.37%). Overall, in all samples, the rate of ARSA was moderate (9.6%) and ASSA at a 9-fold greater rate (89.0%). The highest rate of PCR+ was observed in the laboratory samples (83.3%), while the rate of PCR- in the laboratory samples was 16.7%. By contrast, the rate of PCR+ was very high in hospital samples (96.7%) while the PCR- results were about 30-fold lower (3.3%). Overall the rate of PCR+ samples was very high (94.4%) compared to PCR- samples (5.6%). The number of the specimens collected from female patients exceeded that from male patients in the majority of age groups, i.e., 1-9 years (82.4% vs 17.6%), 10-19 years (55.6% vs 44.4%), 20-29 years (81.9% vs 19.1%), except in the 30-49 years age group, the number of specimens was greater than that of female specimens (70.9% vs 21.9%) and for the age group 50-80 years equivalent sample numbers were collected. Overall, the number of specimens collected from females was also more than collected from males (57.4% vs 42.6%).

The highest rates of OXRSA was found in the newborn age group (71.4%), followed by the 10-19 (66.7%), 30-49 (61.9%), 20-29 (43.8%) and 50-80 (33.3%) year old age groups. The highest rates of OXSSA was found in the 1-9-year old (100%), 50-80-year old (66.7%) and 20-29-year old (56.3%) age groups. Overall, the rates of OXRSA (52.8%) and OXSSA (47.2%) were evenly distributed in the total sample. The only observation of VRSA, i.e., 4.8%, was in the 30–49-year old age group, but VRSA was absent in the other age groups. For VSSA, the rate was 100%. in all age groups, except the 30–49-year age group (95.2%). Overall, the VSSA was higher (98.1%) than the VRSA (1.9%), denoting a low level of resistance to the antibiotic. The highest rate of TRSA was in the age group 20-29-years (81%), followed by 1-9-year old (66.7%), 30-49-year old (38.1%), 50-80-year olds (33.3%), while the lowest rate of TRSA was in newborns (28.6%). The highest rate of TSSA was in the newborn age group (71.4%f 7), followed by the 50-80- and 10-19-year-old (66.7% and 66.7%, respectively) and 30-49-year-old (61.9%) age groups, while the lowest rate of TSSA was in the 20-29-year old age group (18.8%).

EISSA was detected only in the newborn group (14.3%). The highest rate of ERSA was among the age group 10-19-years (100%), followed by the 20-29-year old (81.3%), 30-49-year old (76.2%), 1-9-year old (66.7%) and 50-81-year old (66.7%) age groups. The highest rate of ESSA was in the 1-9-year old (33.3%) and 50-80-year old (33.3%) age groups, whereas in the 30-49-year old age group it was 23.8% and in the 20-29-year old age group it was 18.8%. Overall, the ERSA count was the highest (79.2%), while the ESSA count was 18.9%. GISSA was detected in the 30-49-year old (9.5%) and newborn (14.3%) age groups, whereas GRSA was present in the 1-9-year old (33.3%), 20-29-year old (6.3%), 30-49-year old (14.3%) and newborns (14.3%), but not in the 10-19-year old and 50-80-year old age groups. GSSA was detected at high levels in all age groups, i.e., 1-9-year old (66.7%), 10-19-year old (100%), 20-29-year old (93.8%), 30-49-year old (76.2%), 50-80-year old (100%) and newborns (71.4%). Overall, the ESSA count was the highest (83%) in all samples. The highest rate of KRSA was among the newborn age group (85.7%). Both the 1-9-year old and 10-19-year old age groups

had a KRSA rate of 66.7%, whereas both the 30-49-year old and 50-80-year old age groups had a KRSA of 33.3%. The 20-29-year old age group had a KRSA of 56.3%. In terms of KSSA, the newborn age group had a low rate (14.3%) compared to the rest of the sample, i.e., 33.3% for the 1-9-year old and 10-19-year old, 43.8% for the 20-29-year old, and 66.7% for the 30-49-year old and 50-80-year old age groups. Overall, the rates of KRSA and KSSA were evenly distributed, i.e., 50.9% and 49.1% among the total sample. SRSA was undetectable in the 1-9-year old, 10-19-year old and 50-80-year old age groups, highest in the 20-29-year old (31.3%) and lower in the 30-49-year old (19.0%) and newborn (14.3%) age groups. In the case of SSSA, high rates were detected in all age groups, i.e., 1-9-, 10-19- and 50-80-year old age groups all scored 100%, followed by the newborn (85.7%), 30-49-year old (81.0%) and 20-29-year old (68.8%) age groups. Overall, the SSSA rate (81.1%) also exceeded the SRSA rate (18.9%). Levels of amikacin-intermediate-sensitive S. aureus (AISSA) was undetectable in the 1-9-year old, 10-19-year old, 20-29-year old, 50-80-year old and newborn age groups, but a low level was observed in the 30-49-year old age group (4.8%; 1 out of 21).

ARSA was also absent in samples derived from 1-9-year old, 10-19-year old and 50-80-year old age groups, but was detected in the newborn (42.9%), 20-29-year old (12.5%) and 30-49-year old (4.8%) age groups. ASSA was detected in all samples of the 1-9-year old, 10-19-year old and 50-80-year old (100%) age groups, but also in high levels in the 30-49-year old (90.5%), 20-29-year old (87.5%) and newborn (57.1%) age groups. Overall, ASSA levels (86.8%) were also about 8-fold greater than those for ARSA (11.3%). *S. aureus*-positive strains (PCR+) were identified in all (100%) of samples derived from the 10-19-, 30-49-, 50-80- and newborn age groups, but only in 85.7% of 20-29-year old age group, whereas PCR- strains were identified in the 20-29-year old age group. Overall, most of the samples tested PCR+ for *S. aureus* (96.3%) and 3.7% tested PCR-. The majority of the samples collected from AIH were OXRSA (73.3%) compared with OXSSA (26.7%). In the case of samples collected from CHM and NCIM, the rate of OXSSA exceeded that of OXRSA, i.e., 52.1% and 77.8%, respectively. One sample from MCL tested positive for OXRSA (100%), but no OXSSA was

detected. However, 7 samples from NCIM tested positive for OXRSA (22.2%), while 2 samples from the latter location showed an OXRSA rate of 22.2%. Overall, the combined location rates of OXRSA (50.7%) and OXSSA (49.3%) were evenly distributed. VSSA exceed 90% in samples collected from all locations, i.e., AIH (93.3%), CHM (95.8%), MCL (100%) and MCIM (100%). VRSA rates of 6.7% and 4.2% were observed for AIH and CHM, respectively, but not in the other locations. TRSA was identified in all samples collected from all locations, i.e., AIH (40%), CHM (56.3%), MCL (100%) and NCIM (22.2%). Samples collected from NCIM yielded the highest TSSA rate (77.8%), followed by AIH (60.0%), CHM (43.8%). No TSSA was observed in samples from MCL. Overall, the rates for TRSA and TSSA were equally distributed, i.e., 49.3% and 50.7%. EISSA was detected in only one sample from CHM (2.1%) while the ERSA rate was relatively high in samples collected from all locations, i.e., AIH (73.3%), CHM (70.8%), MCL (100%) and NCIM (88.9%) compared to correspondingly lower ESSA levels, i.e., AIH (26.7%), CHM (27.1%) and NCIM (11.1%). No ESSA was observed in samples from MCL. GISSA rates of 4.2% and 11.1% were observed for samples collected from CHM and NCIM, respectively.

The overall the GISSA rate was low for samples collected from all locations (4.1%). One sample each collected from locations AIH and MCL yielded GRSA at rates of 6.7% and 100%, respectively, whereas samples collected from CHM showed GRSA at a rate of 18.8%. No GRSA was detected in samples from NCIM. The GSSA rates were high in samples collected from AIH (93.3%, CHM (77.1%), NCIM (88.9%), except in samples from MCL for which no GSSA was detected. High rates of KRSA was observed in samples collected from CHM (58.3%) and NCIM (55.6%), and a lower rate in AIH (26.7%), whereas MCL samples yielded no KRSA. Similarly, KSSA was comparatively high in samples derived from all locations, i.e., AIH (73.3%), CHM (41.7%), MCL (100%) and NCIM (44.4%). Overall, the KRSA (50.7%) and KSSA (49.3%) rates were evenly distributed in the sample frame. SRSA was detected in samples collected from all locations, i.e., AIH (40.0%), CHM (6.3%), MCL (100%) and NCIM (33.3%). High rates of SSSA were observed for samples from all locations, i.e., AIH (60.0%),

CHM (93.8%), and NCIM (66.7%), but SSSA was not detectable in samples from MCL. Overall, the SRSA rate (17.8%) was low compared to the SSSA (82.2%) rate. AISSA was detected in one sample collected from AIH (6.7%), but not in samples collected from any of the other locations. ARSA was also not observed in samples collected from MCL, but samples from other locations yielded very low ARSA rates, i.e., AIH (13.3%), CHM (8.3%) and NCIM (11.1%). By contrast, the ASSA was very high in samples sourced from all locations, i.e., AIH (80.0%), CHM (91.7%), MCL (100%) and NCIM (88.9%). Overall, the ASSA rate (89.0%) exceeded the ARSA rate (9.6%). Samples collected from most locations showed PCR+ results, i.e., AIH (100%), CHM (95.7%) and NCIM (100%), but samples collected from MCL all showed PCR- results (100%). Low PCR- results were also detected in samples sourced from CHM (4.3%). Overall, the PCR+ count (94.4%) exceeded the PCR- count (5.6%).

Of the 361 poultry swabs, 19.94% (n=72) showed no bacterial growth, while 80.06% (n=289) showed bacterial growth. The highest number of specimens was collected from Location 5 (36%). Bacterial growth was the highest in swabs taken from under the right thigh (FURT; 21%), followed by swabs taken from under the left thigh (FULT; 19%), FURW (19%; 54 out of 289), from under left wing (FULW; 17%), inside external os of the chicken (IEO; 16%), inside neck after slaughter (INAS; 7%) and from neck after slaughter (FNAS (2%). Of the 48 samples tested for *S. aureus* antibiotic sensitivity, all were sensitive to oxacillin (100%), whereas 96% were sensitive to vancomycin, 35.4% were sensitive to tetracycline and 10.4% showed intermediate sensitivity to tetracycline, 94% were sensitive to gentamicin, 73% to kanamycin, 90% to streptomycin and 100% to amikacin. *S. aureus* antibiotic-resistance were not observed for oxacillin and amikacin, but for vancomycin (4%), tetracycline (54.5%), erythromycin (71%), gentamicin (6%), kanamycin (27%) and streptomycin (10%).

Conclusions

This thesis presents data on the prevalence of MRSA and resistance patterns to other antibiotics in the selected patient specimens from various hospital departments and laboratories as well as

in poultry samples sourced from different locations in Misurata, Libya. Polymerase chain reaction (PCR) was used to verify the presence of *S. aureus* resistance genes (*mecA1*, *mecA2*), and our results suggest that PCR may be a convenient and rapid diagnostic tool for the detection of these lethal microorganisms in patients and livestock animals which may have special public health significance in health care and community settings. The results of this study further indicate that patients and poultry samples were contaminated with *S. aureus* and MRSA. These findings emphasize the need for implementing effective prevention strategies to mitigate the health risk and burden imposed by MRSA colonization. Our results imply injudicious antibiotic prescription and use, non-adherence to or non-existent screening policy or routines, and a general lack of knowledge about multidrug-resistant MRSA strains.

This study presented a local epidemiological situation which should form the basis of empirical antimicrobial therapies at community and health care levels. Further studies are needed to elucidate the transmission routes of MRSA in relation to retail foods and to determine how to prevent the spread of MRSA. MRSA encodes virulence factors which directly transfer between bacteria and serve as an environmental reservoir to trigger pathogenesis. Since the relationship between genetic diversity and virulent factors in MRSA isolates is poorly understood, our study confirms the potential benefit of combining epidemiological and genomic MRSA blood stream infection surveillance to determine the national (indigenous) population structure of MRSA, contextualize previous MRSA outbreaks and identify potentially high-risk strains.

Keywords: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) in humans and poultry, antibiotic resistance profiles, multidrug resistance *mecA1* and *mecA2* genes, altered penicillin-binding protein, polymerase chain reaction (PCR)

LIST OF ABBREVIATIONS

ABR	Antibacterial Resistance
AIH	Assafwa International Hospital
AISSA	Amikacin-Intermediate-Sensitive *S. aureus* | Antibiotic-Intermediate-Sensitive *S. aureus*
AMR	Antimicrobial Resistance
ANT	Aminoglycoside Nucleotidyl Transferase
ARSA	Amikacin-Resistant *S. aureus* | Antibiotic-Resistant *S. aureus*
ASSA	Amikacin-Sensitive *S. aureus* | Antibiotic-Sensitive *S. aureus*
ATCC	American Type Culture Collection
BAC	Blood Agar Culture
BCGN	Bacteria Coagulase-Negative
BCGP	Bacteria Coagulase-Positive
BCN	Bacteria Catalase-Negative
BCP	Bacteria Catalase-Positive
BHIB	Brain-Heart Infusion Broth
BMRSA	Borderline Methicillin-Resistant *Staphylococcus aureus*
BORSA	Borderline Oxacillin-Resistant *Staphylococcus aureus*
BSIs	Bloodstream Infections
CA	Community-Associated
CA-MRSA	Community-Acquired Methicillin-Resistant *Staphylococcus aureus*
CC	Clonal Cluster
CDC	Centers for Disease Control (and Prevention)
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CHM	Central Hospital Misurata

CoNS	Coagulase–Negative *Staphylococcus*
CSF	Cerebrospinal Fluid
DSM	German Collection of Microorganisms and Cell Cultures
DSNCIM	Department of Surgery National Cancer Institute Misurata
ELISA	Enzyme-Linked Immunosorbent Assay
EISSA	Erythromycin-Intermediate-Sensitive *S. aureus*
ERSA	Erythromycin-Resistant *S. aureus*
ESSA	Erythromycin-Sensitive *S. aureus*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
F(t)	Fluorescence as a Function of Time
FDA	Food and Drug Administration (United States)
GBN	Bacteria Gram-Stain-Negative-(*Enterobacteriaceae*+*Pseudomonas*);
GBUND	Growth Bacteria Undulating *Proteus*
GNB	Gram-Negative Bacteria
GISSA	Gentamicin-Intermediate-Sensitive *Staphylococcus aureus*
GPB+	Bacteria Gram-Stain-Positive
GRSA	Gentamicin-Resistant *Staphylococcus aureus*
GS-MRSA	Gentamicin-Susceptible MRSA
GSSA	Gentamicin-Sensitive *Staphylococcus aureus*
HA–	Health care-Associated
HA-MRSA	Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus*
HAI	Health care-Associated Infections/Hospital-Acquired Infections
HARMONY	European Collection of Epidemic MRSA Strains
HGT	Horizontal Gene Transfer
HICPAC	Hospital Infection Control Practices Advisory Committee
HRVISA	Hetero-Resistant VISA
ICU	Intensive Care Unit

IDSA	Infectious Diseases Society of America
KRSA	Kanamycin-Resistant *S. aureus*
KSSA	Kanamycin-Sensitive *S. aureus*
LA-MRSA	Livestock-Associated MRSA
LASER	Light Amplified by Stimulated Emission of Radiation
MCL	Misurata Central Laboratory
MDR	Multidrug–Resistant/Resistance
MDRSA	Multidrug-Resistant *S. aureus*
mecA	Gene Encoding Methicillin Resistance in *Staphylococci*
MGE	Mobile Genetic Elements
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibitory Concentration
MLST	Multilocus Sequence Typing
MLVA	Multilocus Variable-Number Tandem Repeat Analysis
MRCoNS or MR Coag	Methicillin-Resistant Coagulase Negative *Staphylocci*
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin-Susceptible/Sensitive *Staphylococcus aureus*
NARMS	National Antimicrobial Resistance Monitoring System
NCIM	National Cancer Institute Misurata
NCIMDS	National Cancer Institute Misurata Department of Surgery
NCIMICU	National Cancer Institute Misurata Intensive Care Unit
NICU	Newborn Instensive Care Unit
NPV	Negative Predictive Value
nuc a nuc A	Thermostable Nuclease, Unique for *Staphylococcus aureus*
OrfX A	*Staphylococcus aureus*-Specific Gene Sequence

OXRSA	Oxacillin-Resistant *Staphylococcus aureus*
OXSSA	Oxacillin-Sensitive *Staphylococcus aureus*
PBPs	Penicillin-Binding Proteins
PBP2a	An Altered Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PFGE	Pulsed-Field Gel Electrophoresis
PMNs	Human Polymorphonuclear Leukocytes
PPV	Positive Predictive Value
psi	Pounds Per Square Inch or Ψ
PSMs	Phenol-Soluble Modulins
PVL	Panton-Valentine Leucocidin
RAPD	Random Amplification of Polymorphic DNA'
ROC	Receiver Operating Characteristic (Graphic Representation)
SBR	Signal-to-Background Ratio
SCCmec	Staphylococcal Cassette Chromosome *mec*
SD	Standard Deviation
SIRS	Systemic Immune Response Syndrome
SNRL	*Staphylococcus* National Reference Laboratory
SRSA	Streptomycin-Resistant *S. aureus*
SSSA	Streptomycin-Sensitive *S. aureus*
SpaA	Gene Encoding *S. aureus* protein A
TISSA	Tetracycline-Intermediate-Sensitive *S. aureus*
TSA	Tryptic Soy Agar / Trypticase™
TSB	Tryptone Soy Broth
TSSA	Tetracycline-Sensitive *S. aureus*

TRSA	Tetracycline-Resistant *S. aureus*
UTIs	Urinary Tract Infections
VISA	Vancomycin-Intermediate-Sensitive *S. aureus*
VRE	Vancomycin-Resistant *Enterococci*
VRSA	Vancomycin-Resistant *S. aureus*
VSSA	Vancomycin-Sensitive *S. aureus*
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is a multidrug resistant organism that threatens the continued effectiveness of antibiotics worldwide and impedes hospitals in many countries, including Libya (Buzaid et al., 2011). This study investigated the prevalence of MRSA strains and their sensitivity patterns against various antibiotics used for treating patients in hospitals in Musrata, Libya. MRSA should be detected of early for effective prevention and control of the acquired infection (Alzohairy, 2011). MRSA is considered to be more virulent than methicillin-resistant *S. epidermidis* due to the higher biofilm forming abilities of the former to continue decades later and emergency doctors need to be aware of MRSA to provide early medication (Hashem et al., 2017). In addition, outbreaks of infections and new pathogens are potential threats to public health (Carvalho et al., 2010; Marples & Cooke, 1985; Neupane et al., 2018; Shachor-Meyouhas et al., 2018; Zheng et al., 2018).

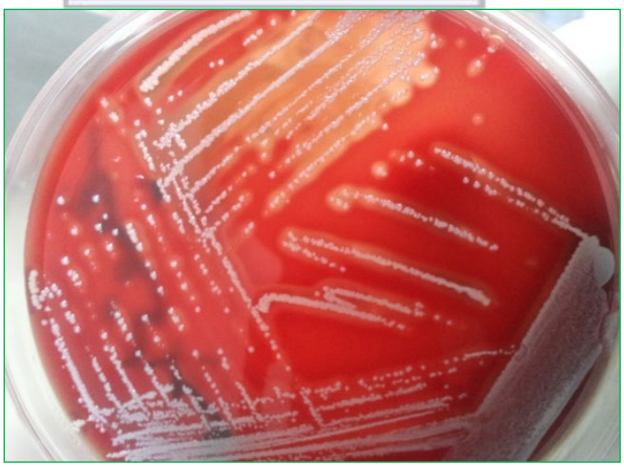
1.2 General Characteristics of *Staphylococcus aureus*

The pathogen, *S. aureus*, is characteristically non-motile, catalase-positive on mannitol salt agar, with facultative anaerobes, and grows in grapelike clusters. This division is based on the production of the enzyme coagulase, enzymes and toxins to decomposition of red blood cells of beta type (haemolysis beta) (Koneman et al., 1997; Nakao et al., 2018; Sasaki et al., 2010; Stegger et al., 2011).The mannitol salt agar (MSA) culture and blood agar culture (BAC) of *S. aureus* are shown in Figures 1.1 and 1.2. *S. aureus*, a bacterial pathogen, is of significant clinical and veterinary importance because it can cause a variety of diseases and because of its capacity to adapt to diverse environmental conditions (Lowy, 1998; Pourramezan et al., 2019;

Price et al., 2012). *S. aureus* is a commensal bacterium that colonises the skin, skin glands and mucous membranes where it can cause invasive, superficial and potentially life-threatening infections, such as sepsis (Ambroggio et al., 2017; de Souza Constantino et al., 2018).

Source: *Staphylococcus aureus* growth and fermentation in mannitol salt agar and its ability to produce yellow colonies Alhussien Ali Elakrout (2014) in the laboratory in Misurata.

Figure 1.1: *Staphylococcus aureus* mannitol salt agar culture



Source: The ability of *Staphylococcus aureus* to secrete enzymes and toxins during decomposition of red blood cells of beta type (haemolysis beta)Alhussien Ali Elakrout (2014) in the laboratory in Misurata.

Figure 1.2: *Staphylococcus aureus* blood agar culture

In addition, these organisms can provid additional protection to skin known as resistance to colonization (Edwards et al., 2010; Percival et al., 2012), endocarditis (Galar et al., 2019;

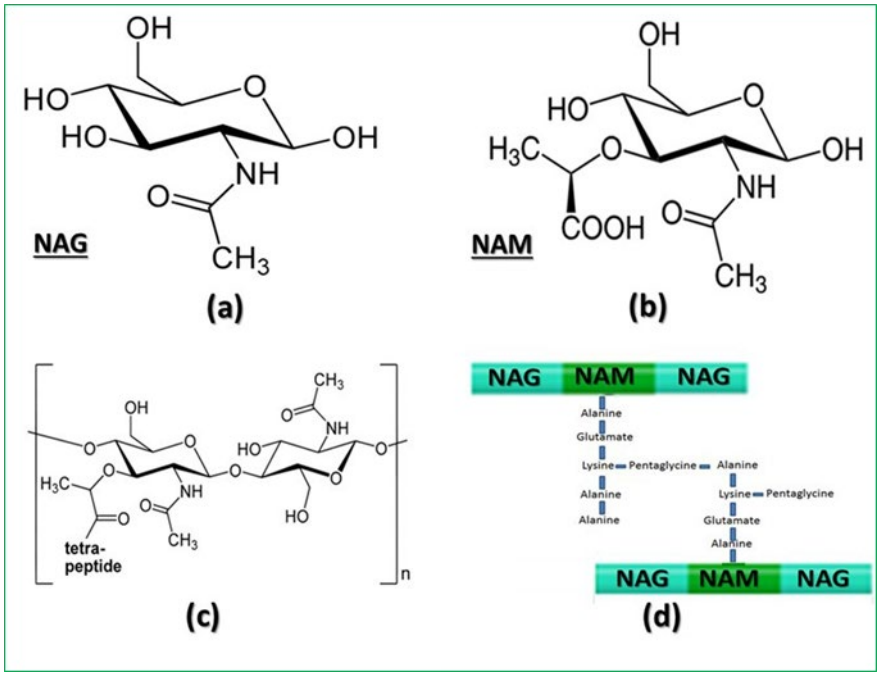
Kitagawa et al., 2019; Yazaki et al., 2018), and necrotising pneumonia (Foster et al., 2014; Leong et al., 2017; Ran et al., 2018). In the 1950s, methicillin, a penicillinase-resistant penicillin derivative, was introduced since penicillin was no longer effective against many infectious *S. aureus* strains. However, within a year after its introduction, methicillin-resistant *S. aureus* (MRSA) strains emerged (Aubry-Damon et al., 1997; Barber, 1961; DeLeo & Chambers, 2009; Hiramatsu et al., 2001; Tong et al., 2008).

Other researchers also found that human *S. aureus* isolates were resistant to the penicillinase-resistant penicillins, more so than animal and product isolates (Wang et al., 2015). The biocidal effect of methicillin on *S. aureus* is the inhibition of the synthesis of the cell wall structure by the group of antibiotics termed penicillins of which methicillin is an example (Rivera & Boucher, 2011; Zhan & Zhu, 2018). Besides, most antimicrobial agents, such as aminoglycosides, were also found to be resistant to the methicillin-resistant isolates (Pelgrift & Friedman, 2013; Szymanek-Majchrzak et al., 2018).

1.3 Cell Wall Composition of *Staphylococcus aureus*

The cell wall consists mainly of peptidoglycan which is based on N-(2,4,5- trihydroxy-6-(hydroxymethyl) oxan-3-yl) acetamide, (N-Acetylglucosamine, N-Acetyl-D- glucosamine, or GlcNAc, or NAG) and 2-(3-acetamido-2,5-dihydroxy-6-(hydroxymethyl)-oxan-4-yl) oxy-propanoicacid (N-Acetylmuramic acid or MurNAc, NAM) (Beveridge, 2001; Yokoyama et al., 2012). Composition of the cell wall of *Staphylococcus aureus* is depicted in Figure 1.3. The first MRSA isolate was probably observed at the Staphylococcus Reference Laboratory in the 1960s (Jevons, 1961).

However, it was only in the 1980s that MRSA appeared to become a significant clinical and epidemiological hospital-associated problem (Lowy, 1998). Many scientists found an underlying mechanism of methicillin resistant protecting bacteria from all β-lactam antibiotics such as penicillin, cephalosporin and carbapenem (Leistner et al., 2015).



Source: In (a) is the structure of N-acetylglucosamine (NAG) (IUPAC: N-((2R,3R,4R,5S,6R)-2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl)acetamide), in (b) is the structure of N-acetylmuramic acid (NAM) (IUPAC: (2R)-2-(((3R,4R,5S,6R)-3-acetamido-2,5-dihydroxy-6-(hydroxymethyl)-oxan-4-yl)-oxy)propanoic acid), in (c), the structure of the polymer and in (d) the peptidoglycan layer (Beveridge, 2001; Carey and Sundberg, 2008).

Figure 1.3: Cell wall composition of *Staphylococcus aureus*

1.4 The Genome Sequencing of *Staphylococcus aureus*

The sequencing of the first *S. aureus* genome was completed in 2001. This revealed the capacity of the organism at its core *vis-a-vis* metabolism, regulation and virulence. Nevertheless, the function of many genes remains unresolved (Cheng et al., 2019; Cunningham et al., 2017; Earls et al., 2017; Madigan et al., 2018). These studies aimed to understand multi-drug resistance and virulence. The outcome was that the location of most of the antibiotic-resistant producing as well as the virulence producing genes was mobile genetic elements (MGEs), exemplified by plasmids, transposons and phosphages. This pointed to considerable horizontal gene transfer from other bacteria.

There are three principal components to the genome structure, which were revealed by comparing this huge amount of genetic data. First, in all strains, there is a backbone of core genes, which are highly (>97%) conserved. Second, throughout the backbone, a group of over

700 core variable (CV) genes are dispersed. Their distribution is variable and the pattern of distribution provides a salient definition of *S. aureus* lineages. Third, are large discrete MGEs that encode the functions of mobilisation, displaying evidence of frequent transfer and, less frequently, recombination (Lindsay, 2014a; Malachowa & DeLeo, 2010). The lineages that infect, and colonise humans, are other lineages found in animals as well and adaptation of major clones. *S. aureus* genomes are highly diverse (Jani et al., 2017; Milheirico et al., 2017). This is partly due to a population structure of conserved lineages (Lindsay, 2014a). The sequencing of the complete genomes of many MRSA strains have recently been published (Chen et al., 2018; Cheng et al., 2019; McClure & Zhang, 2019; Milheirico et al., 2017; Senok et al., 2017).

Comprehensive infection prevention strategies are currently targeting these diverse MRSA strains reduce colonization rates in hospital settings (Mekonnen et al., 2019; Sabbagh et al., 2019; Wong et al., 2018). Genomes are very different due to a population structure of conserved lineages, each with unique combinations of genes encoding surface proteins, regulators and MGEs, which have key proteins for antibiotic resistance, virulence and host-adaptation. MGEs can transfer at high frequency between isolates from the same lineage by horizontal gene transfer (HGT) (Lindsay, 2014a).

Recently, a CRISPR/Cas9 system (pCasSA) for obtained and efficient genome editing, including gene deletion, insertion, and single-base substitution mutation in *S. aureus* has been described. The designed pCasSA system is docile to the assembly of spacers and repair arms by Golden Gate assembly and Gibson assembly, enabling rapid construction of the plasmids for editing. The pCasSA system is an efficient transcription inhibition system for possible genome-wide screening. The CRISPR/Cas9-mediated genome editing and transcription inhibition tools will dramatically accelerate drug-target exploration and drug development and genome editing in *Staphylococcus aureus* (Chen, W. et al., 2017; Liu et al., 2017).

1.5 Pulse-Field Gel Electrophoresis (PFGE)

PFGE is the best method to identify *Staphylococcus aureus* and different strains of bacteria (Bernardo et al., 2002; Murchan et al., 2003; Olive & Bean, 1999). Antibiotic sensitivity tests, molecular typing by PCR and PFGE are often used in combination to identify community-associated MRSA (Jain et al., 2019; Murai et al., 2019; Pereira-Franchi et al., 2019; Uehara et al., 2019). Thirty-two isolates of CG-MRSA were investigated, from four cities in Colombia, South America., by using gel electrophoresis, SCCmec, agar and spa typing, and whole-genome sequencing, were all isolates belonged to ST923, harbouring SCCmec IVa and a spa type t1635 and lacked an arginine catabolism mobile element. The isolates were classified as COL923 which were resistant to most antibiotics, of which most (>60%) showed resistance to macrolides and tetracycline (Escobar-Perez et al., 2017).

For *S. aureus*, the method depends on an analysis of crumbs of SmaI-digested *S. aureus* genomic DNA. The performing banding patterns are analyzsed using a special software package, such as GelCompar II of Applied Maths using Dice comparison and unweighted pair matching analysis (UPGMA). Regulation is according to established criteria (Burke et al., 2004; Tenover et al., 1995). *Spa* typing of MRSA strains was compared to phage typing and, in general, concordance was found between the two methods, that is Spa typing was more sensitive (Omar et al., 2014).

1.6 Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection

Invasive MRSA infection affects certain populations excessively, and it is a major public health menace primarily related to health care, though no longer confined to intensive care units, acute care hospitals, or any health care institution and, also Gram-negative bacteria (MDR-GNB) are a leading cause of morbidity and mortality worldwide (Majelan et al., 2019; Regev-Yochay, 2019; Tacconelli et al., 2014; Upreti et al., 2018). MRSA has been a public health problem since the 1960s, although community outbreaks of MRSA occur in different populations, including American Indians and Alaskan Natives (Baggett et al., 2004).

Clonal complex 5 methicillin-resistant *Staphylococcus aureus* (CC5-MRSA) comprises numerous prevalent clones that cause hospital-associated infections in the Western hemisphere, and the correlations of different clades and clones of CC5 implicate genomic alterations for increased antibiotic resistance and decreased virulence associated with the spread of these MRSA strains (Challagundla et al., 2018). The prevalence of MRSA isolates varies greatly among countries in Africa, however, it appears to have soared since 2000 in many African countries, except in South Africa (Falagas et al., 2013). The drug of choice for treatment of MRSA is penicillin, but resistance to the antibiotic was recognized in many countries as early as 1940 (Abraham & Chain, 1940), while penicillinase-producing strains were also reported (Kirby, 1944) when antibiotics then used in an irregular manner led to the emergence of multidrug-resistant pathogens (Medina & Pieper, 2016).

1.7 Diagnosis and Identification of *Staphylococcus aureus*

Microscopically, one can characterize *Staphylococcus aureus* as clusters or pairs of Gram-positive cocci, using the catalase, coagulase and mannitol salt agar (MSA) culture and DNase enzyme tests (Becker et al., 2003; Gilligan, 2013; Kateete et al., 2010; Koneman et al., 1997; Saputra et al., 2017; Sperber & Tatini, 1975), and also MRSA identification by phenotypic and genotypic assays (Fluit et al., 2001). This bacterium is catalase-positive, non-sporing and non-motile, and due to its production of catalase, it may be differentiated from *Streptococci* and other Gram-positive bacteria (Kloos & Bannerman, 1995; Kloos & Schleifer, 1986). The bacterium produces lactic acid by fermenting glucose (Mathew et al., 2017).

Agar media, such as MSA, a selective media containing 7-9% NaCl, has been used. This media favours the growth of *Staphylococcus aureus*, and golden-yellow colonies arise, due to mannitol fermentation (Azuure, 2016; Safdar et al., 2003). In order to identify *S. aureus*, molecular-based tests have also been used; these involve the use of the Polymerase Chain Reaction (PCR) (Al-Talib et al., 2009; Herma et al., 2017; Milheiriço et al., 2007; Ogihara et al., 2018; Rahman et al., 2018; Sadeghi et al., 2019; Seki et al., 2015). This test has been

formulated to be species-specific, and to amplify specific target sites (Ajdler-Schaeffler et al., 2018; Reddy et al., 2017). A commercially available kit, which is real-time, has been constructed to identify *S. aureus*. It detects specific sequences of the bacterium within the internal transcribed spacer (ITS) of the *S. aureus* region (Harbarth et al., 2011; Levi & Towner, 2005; Yam et al., 2013).

The use of blood cultures in the diagnosis of *S. aureus* infections is especially valuable in cases such as endocarditis and bacteremia, i.e., in situations of deep-tissue infections (Dark et al., 2009; Stefani, 2009). It is advisable to couple blood culture tests with other identification or diagnostic tests when critically ill patients are involved, because of the slowness of producing blood culture results (Harbarth et al., 2003). Another useful test is the DNase enzyme test (Madison & Baselski, 1983). Other similarly productive tests are the lipase test (with a yellow colour and rancid odour) and the phosphatase test with a pink colour (Philp et al., 1997). One may cite here also other identification tests, such as the latex agglutination tests that detect various surface antigens, such as protein A and other clumping factors. In these tests, cross-reactivity with CoNS may compromise specificity. Moreover, some *S. aureus* stains may not produce the clamping factor or protein (Koneman et al., 1997).

1.8 Protein A Gene Typing of *Staphylococcus aureus*

Staphylococcus aureus isolates are typed through DNA sequence analysis of the X zone of the protein A gene (*Spa*). Also, *spa* typing is compared for both phenotypic and molecular techniques for the ability to distinguish and categorize *S. aureus* strains into series that correlate with epidemiological information (Asadollahi et al., 2018; Shopsin et al., 1999). Due to the high variability in *Spa* types, researchers collected 1,536 MRSA isolates of 319 patients during a five-year period and found *Spa* type alterations in 30 MRSA isolates; the alteration most often seen was the deletion of repeats, followed by repeat duplication and point mutation (Boye & Westh, 2011). The new PCR-based method of typing *Staphylococcus aureus* was compared to pulsed-field gel electrophoresis (PFGE) and it was found that *Spa* typing has a

discriminative power between that of PFGE and multilocus sequence typing (MLST) (Malachowa et al., 2005). Current DNA expansion-based typing methods for bacterial pathogens mostly lack inter-laboratory reproducibility for DNA sequence-based typing of the *Staphylococcus aureus* protein A gene (spa, 110 to 422 bp) (Aires-de-Sousa et al., 2006).

1.9 Toxins Produced by *Staphylococcus aureus*

Depending on the strain, *S. aureus* secretes exotoxins and enterotoxins that are grouped into three: superantigens, exfoliative toxins, and other toxins. People with diabetes, and those in crowded spaces, like boarding school dormitories, prisons facilities, injection drug users, and patients with infections using catheters, are at risk, making them susceptible to becoming infected with *S. aureus* (Basanisi et al., 2017; Becker et al., 2003; Horiuchi et al., 2019; Huang & Chou, 2019; Lin et al., 2017; Mulvey et al., 2005; Pereira-Franchi et al., 2019).

1.9.1 Virulence

S. aureus and MRSA exhibit virulence that is multifactorial, i.e., their virulence depends on a multitude of toxins, surface proteins, strategies that are immune-evasive and other hallmarks of virulence. The toxin repertoire of the infecting strain largely determines the severity of the infection by *S. aureus* (Queck et al., 2009; Reddy et al., 2017). Examples of toxins and super antigens (entertoxins) produced by *S. aureus* are toxic shock syndrome toxin-1 (TSST-1) (El-Ghodban et al., 2006; Maeda et al., 2018; Miura et al., 2018; Reddy et al., 2017), leukocidins (Konig et al., 1995; Reddy et al., 2017; Sun et al., 2017; Taneike et al., 2006), α-toxins, and phenol-soluble modulins (PSMs) (Jang et al., 2017; Queck et al., 2009; Wolfmeier et al., 2018). Thus, the capacity of *S. aureus* to evade immune systems is greatly increased by these molecules, as they destroy immune cells.

1.9.2 Panton Valentine Leucocidin

The two toxins that comprise the Panton Valentine Leucocidin (PVL) toxin are lukS-PV and LukF-PV. These component toxins are from a family that form a barrel of cytolytic toxins

which, in turn, comprise several other leukocydins and toxins (Otto, 2013). This is confirmation of the considerable impact of PVL on CA-MRSA virulence in several animal models. Several studies in experimental animal models indicate that there is little or no contribution of MGEs to the virulence of the CA-MRSA strain, with the exception of cases of necrotising pneumonia and possibly in the early stages of skin infections. This finding remains perplexing, despite the attempt by many initial studies to link the phenotype of enhanced virulence to MGEs (DeLeo et al., 2010; Diep et al., 2010).

1.9.3 Phenol-Soluble-Modulins (PSMs)

The most toxic PSMs are the smaller type-αPSMs. These are PSM-α1, PSM-α2, PSM-α3 PSM-α4 and those α-toxins with a length of 20-30 amino acids (Wang et al., 2007). Those with an approximate length of 44 amino acids are the larger type-α PSMs (PSMα and PSMα2) and have additional functions, such as contributing to the structure of biofilms and the propagation of inflammation (Wang, R. et al., 2011). PSM-mec belongs to the PSM class of *S. aureus* peptide toxins, yet stands in contrast to all other known PSMs. This contrast is manifested in its being encoded on MGEs, more specifically on SCCmec elements of types 23 and 8. Hence, one observes thereby a correlation with MRSA lineages (Chatterjee et al., 2011; Queck et al., 2009). It has been shown that *psm-mec* share the characteristic *Agr*-dependent, yet RNAIII-independent, regulation with the other core-genome encoded psm genes (Chatterjee et al., 2011). In CA-MRSA strains, one observes that high amounts of PSMs are produced, yet on the other hand, on average, production of the same is lower in typical HA-MRSA strains, such as USA100 and USA200 (Wang et al., 2007).

1.9.4 Alpha-Toxins

S. aureus releases cytotoxins of which the alpha-toxin or alpha-haemolysin is the prototype. The toxin, a member of the pore-forming beta-barrel toxin family, was the first to be identified, mostly comprising beta sheets. Erythrocytes and macrophages were found to be sensitive to haemolysis through alpha-toxin and its interaction with the receptor, A-Disintegrin and

Metalloproteinase (ADAM10), seemed to disrupt the epithelial cell barrier function which demonstrated that ADAM10-deficient mice were protected from lethal pneumonia and severe *S. aureus* skin infection (Berube & Wardenburg, 2013). Alpha-toxin also increased virulence by enabling *S. aureus* pneumonia through CXC chemokine gradients and stimulating chemokine-induced neutrophil chemotaxis (Bartlett et al., 2008). In addition, inflammasomes promote the pro-inflammatory cytokines Interleukin-1β (IL-1β) and Interleukin-18 (IL-18) of alpha-toxin (Craven et al., 2009). The taxonomy of the pathogen is given in Table 1.1.

Table 1.1: Taxonomy of *Staphylococcus aureus*

Domain	**Bacteria**
Kingdom	*Eubacteria*
Phylum	*Firmicutes*
Class	*Bacilli*
Order	*Bacillales*
Family	*Staphylocaeae*
Genus	*Staphylococcus*
Species (cause of human disease)	*S. aureus*
	Epidermis
	Saprophyticus
	S. haemolyticus
	S. lugdunensis

1.10 *Staphylococcus aureus* Food Poisoning

Staphylococcus is the most virulent cause of human poisoning and is transmitted by food-borne bacteria. More than twenty SEs have been described: SEA to SElV. All of them have super-antigenic activity, whereas half of them have been proved to be emetic, representing a potential hazard for consumers. This can be divided into four parts, (1) the worldwide story of SFP outbreaks, (2) the characteristics and behaviour of *S. aureus* in the food environment, (3) the

toxinogenic conditions and characteristics of SEs, and (4) SFP outbreaks, including symptomatology (Hennekinne et al., 2012). Another study in Egypt confirmed that chicken meat and its products were considered as an important source of spreading of MRSA in humans. Strict hygienic measures must be taken in food preparing establishments and in poultry slaughter (Karmi, 2013). For MRSA, the primary factor of acute health care-associated infection (HAI) and community-associated MRSA (CA-MRSA) infections has arisen in the general populace. Moreover, CA-MRSA, livestock-associated MRSA (LA-MRSA) and HA-MRSA (HA-MRSA) are also found in foods intended for human consumption, especially animal-origin foods, and may involve livestock, as well as humans involved in animal husbandry and food-processing (Igbinosa et al., 2016; Sergelidis & Angelidis, 2017).

1.11 Treatment Rationales for *Staphylococcus aureus* Infections

Through the production of beta-lactamases, mutation of the normal penicillin-binding protein, and/or acquisition of the *mecA* gene that encodes for an alternative penicillin-binding protein, MRSA are cross-resistant to beta-lactam antibiotics, including oxacillin, nafcillin, dicloxacillin and cefazolin. Benzylpenicillin, known as penicillin G, was discovered in 1928 by the Scottish scientist and Nobel laureate, Alexander Fleming. Penicillin and its derivatives, which include methicillin, is used as treatment for infections caused by *S. aureus* (Rayner & Munckhof, 2005).

Penicillin was initially highly effective against *Staphylococcal* infections, but penicillinase-produced by *S. aureus* emerged in the mid-1940s (Kirby, 1944) to limit its potency, and so a combination of the penicillin-derived semi-synthetic antibiotic ampicillin and another chemical, sulbactam, gave rise to ampicillin/sulbactam to overcome such resistance. This combination was introduced in 1987 as an intravenous antibiotic under the trade name, Unasyn, in the USA. Oxacillin-resistant *Staphylococcus aureus* (OXRSA) has now increased in prevalence (Anurag Payasi, 2015; Basset et al., 2010; Fridkin et al., 2002; Holden et al., 2013). Synthetic fluoroquinolone, Moxiflocacin, is a third-generation oral antibiotic with the trade

name, Avalox, which was approved in the USA for life-threatening infections in 1999 after being submitted for approval ten years earlier. This broad-spectrum antibiotic works by inhibiting DNA gyrase, an enzyme in topoisomerase II and IV, needed for replication, transcription, and recombination (Drlica & Zhao, 1997). Cystitis and bacterial urinary tract bacterial infections are usually treated with ciprofloxacin, whereas clindamycin, a lincosamide antibiotic is used to treat anaerobic bacterial infections (Kaddora, 2010). As bacteriostatic and bactericidal antibiotics, oxacillin or flucloxacillin, despite reports of resistance (Bai et al., 2019; Looney et al., 2017; Pardos de la Gandara et al., 2018), have been used in first-line therapy and are penicillinase-resistant beta-lactam antibiotics to treat serious infections, such as endocarditis (Bayer et al., 1998; Galar et al., 2019; Korzeniowski & Sande, 1982)—a combination therapy with gentamicin is given. However, the use of gentamicin poses some controversy as its use can lead to kidney damage (Cosgrove et al., 2009). Vancomycin is considered as the best treatment option in MRSA infections (Anurag Payasi, 2015; Chavada et al., 2017; Yoon et al., 2014). However, its downside includes absorption in the gastrointestinal tract which is rather poor, slow bactericidal activity and many other side effects (Gould, 2008; Levine, 2006; Levine et al., 1991).

1.12 Molecular Epidemiology of Resistant *Staphylococcus aureus*

1.12.1 Nosocomial Methicillin-Resistant *Staphylococcus aureus* (MRSA)

S. aureus, a substantial contributor behind infection that occurs in hospitals and the highest proportion of deaths was by MRSA (Joo, 2019; Jorgensen et al., 2019; Kim et al., 2019; Nelson et al., 2017; Sit et al., 2018; Uematsu et al., 2017). MRSA is one of the key causes of bovine mastitis which can be transmitted from animals to humans through drinking milk if this is not treated (Ahangari et al., 2017). First, MRSA must be detected and identified in hospitals. Nevertheless, it has since been detected in the community as well as in livestock with a heightened global prevalence rate as reported in European countries (Stefani et al., 2012). MRSA is noticeably known in hospital settings worldwide, based on this comparison, infection

in the United States of America between the years 2011 and 2015; in 2011 fewer infections occurred between Inpatients than among persons in the community without recent health care exposures (Dantes et al., 2013). The term MRSA is used instead of hospital-acquired infection (HAI) for *S. aureus*, and repeatedly, HAI is also used to refer to health-acquired infections and has a higher, approximately double, attributable death rate, compared to that of methicillin-susceptible infections (Blot et al., 2002; Hurley, 2002; Whitby et al., 2001). Notably, 40.4% of HA, 25.5% of CA *S. aureus* infections, 67.4% HA infections, which was CA-MRSA isolates from ST59-MRSA-SCCmec kind, IV-spa kind t437, ST72-MRSA-SCCmec kind IV-spa kind t324 and ST30 MRSA-SCCmec kind IV-spa kind t019 (Song et al., 2011).

MRSA is the reason behind the CA-S of the inflammation, as molecular analysis of CA MRSA isolates to strain diversity with USA 300 and disease symptoms are constrained with changes in CAS, these are to some extent, strain specific changes in the CA-*S. aureus*, in part, and are related to changes in immunity to the USA300 clone (Hultén et al., 2018). This represents strains from MRSA infection S-239, about 90% are from the MRSA infections in Asian countries, such as China, where it is highly resistant to antibiotics (Xu et al., 2009).

The study between 2016–2017 among 239 podiatrists in Spain showed the prevalence of *S. aureus* and *S. epidermidis* nasal carriage is low among Spanish podiatrists compared to other health professionals (de Benito et al., 2018) and showed the importance of host, pathogen and treatment characteristics in determining short-term or in-hospital mortality for patients with MRSA (Guillamet et al., 2018). By contrast, for the rise in levels of PFGE difference displayed by ST239 strains, almost all isolates carry the *SCCmec* type III genetic element, which is a composite element of about 67 kb that confers resistance to methicillin and other antimicrobials (Smyth et al., 2010). Hospital-acquired infections are a major cause of morbidity and mortality in neonatal intensive care units (NICU) (Chen, B. J. et al., 2017). New evidence shows that the CC8-ST239-III-t037 lineage in South Korea, Hong Kong, Taiwan and Vietnam and the CC5-ST5-II-t002 lineage in South Korea and Sri Lanka have spread from hospitals to the

community and, epidemiologically at least, have become regarded as CA-MRSA (Song et al., 2011; Stefani et al., 2012). The diffusion of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) into hospitals has been recurrently reported globally (Berla-Kerzhner et al., 2017; Edslev et al., 2018; Feng et al., 2017; Paternina-de la Ossa et al., 2018; Thurlow et al., 2012; Wan et al., 2019). Hospital-acquired MRSA (HA-MRSA) strains reveal high-level resistance to multiple antimicrobial agents, whereas CA-MRSA strains are commonly subjected to non-β-lactams.

It is predicted that the antibiogram of the HA-MRSA population would change along with the change in genotype of MRSA (Harada et al., 2018). CLABSI incidence rates as a comparator to examine the impact of MRSA guidelines in Québec adult hospitals from January 1st 2006 to March 31st, 2015, can be seen by looking at the incidence rate reduction (IRR) in health care-associated MRSA bloodstream infections (HA-MRSA) (Li et al., 2017); many community-associated MRSA (CA-MRSA) infections increase in otherwise healthy individuals who do not have such risk factors. Further, CA-MRSA infections are epidemic in some countries. These features denote that CA-MRSA strains are more virulent and transmissible than are traditional hospital-associated MRSA strains (DeLeo et al., 2010). Given the high population numbers in the countries where it prevails, ST239 may represent the most successful MRSA lineage around the world (Uhlemann et al., 2014). MRSA in prisons may cause serious morbidity and death with rates and risk factors for MRSA infection in custody (Haysom et al., 2018).

1.12.2 CA-MRSA Strains Can Be Distinguished from HA-MRSA Strains

CA-MRSA clones, such as ST8 USA300, ST30, ST59 and ST80, have been spreading rapidly in the community and are now also infiltrating health care settings in many regions around the world (Stefani et al., 2012). This follows the emergence of hVISA bloodstream infections (BSIs) by Etest® macromethod among patients with a non-hVISA BSI at baseline (Martirosov et al., 2017). There are limited data on MRSA carriage in dental clinics, however, recently

1300 specimens from patients, health personnel, and environmental surfaces of a dental clinic in Egypt were examined for MRSA, and the results indicate high MRSA pathogenicity in dental wards underscoring the dire need for more efficient surveillance/infection control strategies (Khairalla et al., 2017). In asylum seekers, between January 1st 2014 and December 31st 2015, testing for MRSA and for multidrug use, results compared these with cultures from the Dutch patient population with risk factors; a total of almost 10% were MRSA positive. Of 118 asylum seekers with *S. aureus* in clinical cultures, almost 19% were MRSA positive (Ravensbergen et al., 2017).

Staphylococcus aureus causes many diseases and infections, including soft-tissue infections (SSTI), skin infections, endovascular infections, septic arthritis, pneumonia, endocarditis and osteomyelitis (David & Daum, 2010). A study in Australia revealed Type V SCCmec is a small SCCmec element (28 kb) and does not carry any antibiotic resistance genes. A fifth all type of SCCmec was found on the chromosome from a community-acquired methicillin-resistant *Staphylococcus aureus* strain (strain WIS [WBG8318]) (Ito et al., 2004).

Infections from MRSA have caused serious problems in society as they are mostly infections in the skin, especially between children (Fridkin et al., 2005). They were discovered for the first time in Malaysian hospitals and were the emergence of MRSA clones, such as SCCmec type I-ST152, SCCmec type V-ST45ostly and SCCmec type V-ST951, that correlated the clinical, phenotypic and genotypic characteristics of patients with MRSA bacteraemia, as well as determined the risk factors for mortality in Malaysian hospitals (Medina Cruz et al., 2018). CA-MRSA strains can be distinguished from HA-MRSA strains based on three characteristics with respect to antibiotic resistance:

1. Firstly, CA-MRSA strains host different types of SSCmec elements, most frequently types IV and V compared to types I, II, and III in HA-MRSA. The recently adopted opinion is that type IV and V SSCmec elements, because of their smaller sizes

compared to the other SSCmec elements, may be associated with lower fitness costs (Daum et al., 2002);

2. Secondly, CA-MRSA isolates are usually sensitive to most antibiotics, excluding methicillin and β-lactams, while multi-resistance is common in HA-MRSA isolates (S Naimi et al., 2004); and

3. Thirdly, minimal inhibitory concentration MIC values of HA-MRSA clones are usually higher than those of CA-MRSA clones.

There are, currently, two theories that have been suggested to explain increased CA-MRSA virulence.

1. The first hypothesis attributes increased CA-MRSA virulence to the acquisition of MGEs, i.e., those containing Panton-Valentine leucocidin PVL (Vandenesch et al., 2003).

2. Secondly, CA-MRSA virulence is explained by increased expression of core genome-encoded virulence genes, such as phenol-soluble modulin PSM cytolysins, α-toxin and other virulence determinants (Li et al., 2009).

These are spreading rapidly in the community and are now also finding a way into health care settings in many regions of the globe (Stefani, et al., 2012). CA-MRSA clones, such as ST8 USA300, ST30, ST59 and ST80, have been spreading.

1.12.3 Aminoglycoside Resistance

Data from 236 patients were analyzed to identify the relationship between plasma aminoglycoside concentrations, the minimal inhibitory concentration (MIC) to the infecting organism and therapeutic result (Moore et al., 1987). Amino acid sequences were compared

with 49 enzymes and have revealed new insights into the evolution and relatedness of these proteins. This applies to the amino acids which may be important in binding, also the layers of enzymes which inactivate aminoglycoside while leading to bacterial resistance, are reviewed (Shaw et al., 1993). It was found one aminoglycoside was in 66.7% of HA-MRSA and in more than 22% of all of them. The presence of the aacA-aph D gene was sufficient to express the resistance phenotype to GEN/TOB/AK/NET. Resistant isolates were closely related (Szymanek-Majchrzak et al., 2018). This is the first time the anti-MRSA synergism of prenylflavonoids 1-4 with eleven antibacterial agents and the reflex of MRSA resistance to aminoglycosides, particularly amikacin has been observed. The results might be valuable for the development of new antibacterial drugs and synergists against MRSA infection (Zuo et al., 2018). There is a diversity of MRSA aminoglycoside-resistance genes that may be exploited to target for the effective treatment of infections due to MRSA strains (Khosravi et al., 2017).

1.12.4 *Staphylococcus aureus* Resistance to β-Lactam Antibiotics

The ß-lactam antibiotics are considered the most generally used antimicrobial agents to kill bacteria and thus to treat infections (Foster, 2017; Van Boeckel et al., 2014). The ß-lactam antibiotics exert their bactericidal activity by inhibiting enzymes that participate in bacterial cell-wall synthesis, i.e., penicillin-binding proteins (PBPs). The 78-kDa PBP (known as PBP2a or PBP2', confers resistance to methicillin and cross-resistance to other β-lactam antibiotics. PBP2a is an alternative transpeptidase that has low affinity for β-lactam antibiotics and is able to catalyse cell-wall synthesis, even when normal PBPs are covalently linked to β-lactams (Brown & Reynolds, 1980; Figueiredo & Ferreira, 2014).

ß-Lactamases, the bacterial enzymes that hydrolyze ß-lactam antibiotics, present a major impediment to the successful treatment and prophylaxis of almost all infectious diseases (Bonomo, 2017; Pratt, 2016). MRSA is the principal cause of persistent infections in humans, including endocarditis, pneumonia, and toxic shock syndrome (Gillard et al., 2018; Zhan & Zhu, 2018). Therefore, more efficacious therapeutics and modalities are deemed necessary to

eradicate the scourge of MRSA and other resistant bacteria, including inhibition of biofilm formation to treat diseases associated with MRSA infection (Zha et al., 2019), novel vancomycin and cefazolin nanoplexes to improve drug delivery to combat MRSA infections (Hassan et al., 2019; Nicolau & Silberg, 2017), computational analysis of the interactions of novel cephalosporin derivative with beta-lactamases (Verdino et al., 2018), delafloxacin—a novel oral and intravenous fluoroquinolone with activity against MRSA and *Pseudomonas aeruginosa*, advancing new options for the treatment of acute bacterial skin and skin-structure infections (ABSSSIs) and complicated urinary tract infections and severe community-acquired bacterial pneumonia (Ocheretyaner & Park, 2018) and plant-derived bioactive compounds with anti-MRSA modalities of plant antimicrobials such as inhibtion of in efflux pump activity, inhibition of pyruvate kinase and disturbance of quorum sensing in MRSA (Li et al., 2018; Saddiq & Al-Ghamdi, 2018). The literature abounds with many promising and futuristic alternatives to the current arsenal of ß-lactam antibiotics targeting MRSA and its virulence factors (Milheirico et al., 2017; Muteeb et al., 2017; Ni et al., 2017; Tharmalingam et al., 2017; Vaishampayan et al., 2018; Wang et al., 2007; Waters et al., 2017; Yang et al., 2017).

1.12.5 *SCCmec* and *mecA* Genes

S. aureus (MRSA) strains contain large, but not homologous MGEs called the staphylococcal cassette chromosome mec (*SCCmec*) and the gene encoding methicillin resistance in Staphylococci (*mecA*), the major elements of methicillin resistance (Figueiredo & Ferreira, 2014; García-Álvarez et al., 2011; Jones et al., 2018; Zhan & Zhu, 2018). *SCCmec* is responsible for vertical and horizontal transfer of methicillin resistance (Stojanov et al., 2012). Resistant *S. aureus* might spread from the patients to the community (Edslev et al., 2017). The *mecA* gene is also a mobile genetic element which encodes a methicillin-insensitive transpeptidase, to give resistance to otherwise inhibitory concentrations of ß-lactam antibiotics (Xu et al., 2018; Zhan & Zhu, 2018). The *Staphylococcus aureus* MRSA contains the mec gene complex (which comprises the *mecA* gene and its regulators *mecI* and mecR). SCCmec also

contains the *ccr* compound, which encodes the site-specific control *ccrA* and *ccrB*, which transfers *SCCmec* between staphylococci (Katayama et al., 2000). MRSA ST22 SCC mec IV has been isolated from livestock or primates (Roberts et al., 2018). The *mecA* gene on SCCmec is responsible for vertical and horizontal transfer of methicillin resistance (Stojanov et al., 2012).

1.12.6 Community-Associated MRSA (CA-MRSA)

It is thought that MRSA originally emerged in nosocomial settings and has subsequently spread into the community. In turn, community-associated MRSA (CA-MRSA) lineages are re-introduced from the community into hospitals where they can cause hospital-associated MRSA (HA-MRSA) infections (Copin et al., 2019; Hogan et al., 2019; Mekonnen et al., 2019; Penteado et al., 2019; Pham et al., 2018; Rokney et al., 2019; Song et al., 2018). Environmental resevoir MRSA (residual MRSA) is one of the most important causes of nosocomial infections worldwide as demonstrated by the global prevalence of MRSA since the 1960s (Chatterjee & Otto, 2013; Mitchell et al., 2014). MRSA is a risk factor in hospitals worldwide and causes substantial morbidity and mortality. Health-care-associated MRSA (HA-MRSA) infections arise in individuals with predisposing risk factors, such as surgery or the presence of an in-dwelling medical device; the CA-MRSA strains are more virulent and more transmissible of the HA-MRSA strains (DeLeo et al., 2010).

Staphylococcus aureus is a well-defined risk factor of infection with these bacteria and is increasingly dangerous in the nose and its transmission to other persons. There is evidence for the detection of nasal carriage of *S. aureus* in patients undergoing cardiac surgery and in those undergoing hemodialysis (Verhoeven et al., 2014). The RAND/UCLA method is used to detect, in infectious diseases, the role of glycopeptides in the management of MRSA infections, bacteraemia and endocarditis, joint replacement infections, skin and soft tissue infections, diabetic foot, abdominal infections and central nervous system infections (Concia et al., 2018). CA-MRSA infections have also been linked to severe invasive diseases, such as necrotising

pneumonia, and prone positioning in treating severe respiratory failure caused by community-acquired infection may minimise the risk of barotrauma, and provide better drainage of secretions in patients with necrotising pneumonia (He et al., 2017). MRSA clones such as SCCmec type I-ST152, SCCmec type V-ST45 and SCCmec type V-ST951 were discovered for the first time in Malaysia, correlating with the clinical, phenotypic and genotypic characteristics of patients with MRSA bacteraemia, as well as determining the risk factors for mortality in Malaysian hospitals (Sit et al., 2018). Staphylococcal cassette chromosome (SCC) mec typing showed that the predominant HA-MRSA strains in the hospital dramatically changed from SCCmec type II, which is the major type of HA-MRSA, to SCCmec type IV, which is the major type of CA-MRSA. Multilocus sequence typing revealed that the predominant SCCmec type IV strain was a clonal complex (CC) 8 clone, which is mainly found among CA-MRSA (Harada et al., 2018).

1.12.7 Livestock-Associated MRSA (LA-MRSA)

During 2010–2011, within the framework of the National Antimicrobial Resistance Monitoring System (NARMS, https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/about-narms#NARMS), MRSA was detected in meats. Retail meat samples (ca. 3520) were collected from eight U.S. states, 27.9% contained *S. aureus* and 1.9% were positive for MRSA; about 10.4% of *S. aureus* isolates, including 37.2% of MRSA, were multidrug-resistant *S. aureus* (MDRSA) (Ge et al., 2017). Several similar reports of the prevalence of MRSA in animal and food products have appeared (Logue & Andreasen, 2018; Oniciuc et al., 2017), including livestock (Lee, 2003), along the production chain of dairy products in north-western Greece (Papadopoulos et al., 2018) and Italy (Carfora et al., 2015), milk obtained from culled dairy cows and from cows with acute clinical mastitis (Ismail, 2017), Turkish cheeses (Can & Çelik, 2012), poultry and poultry meat (Ali et al., 2017; Ribeiro et al., 2018), along the pig slaughter line (Vossenkuhl et al., 2014), companion and food-chain animals (Vossenkuhl et al., 2014) and even workers in the food industry (Strommenger et al.,

2018; Zarazaga et al., 2018). Genotyping of MRSA isolates prevalent in intra-mammary infections in dairy cows revealed *SCCmec* cassettes that were classified as type IV, type V or type IV/V composite. All or most strains harboured the genes encoding the β-lactamase operon and tetracycline resistance (Luini et al., 2015). In New Zealand, twenty-two cases of LA-MRSA were detected, 4 persons reported contact with sheep and cattle; 2 of these persons lived on farms with livestock positive to *mecC*-carrying MRSA, sharing *spa* type (t843), and MLVA (MT429) and PFGE pattern for the human isolates. These observations indicate that *mecC*-carrying MRSA can be exchanged among humans and ruminants (Petersen et al., 2013). Animals can act as reservoirs of MRSA, and the source of bacterial pathogens with the potential to be transmitted to people in close contact with the animals have major public health implications for colonised animals (Abd El-Hamid et al., 2019; Becker et al., 2017; Bortolami et al., 2017; Chen & Huang, 2018).

1.12.8 MRSA in Libya

It is widely accepted that the burden of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Multidrug-Resistant *S. aureus* (MDRSA) is a global public health problem, and Libya is no exception (Ahmed et al., 2017; Ali et al., 2014; BenDarif et al., 2016; Frickmann et al., 2018; Khemiri et al., 2017; Mathlouthi et al., 2016; Ouertani et al., 2016; Shittu et al., 2018; Sifaw Ghenghesh et al., 2013; Zorgani, A. et al., 2015). PCR detection of toxic shock syndrome toxin-1 (TSST-1) in 63 *Staphylococcus aureus* strains (40 from clinical sources and 23 from food sources) has been reported from Libya more than a decade ago (El-Ghodban et al., 2006). Zorgani et al. (2006) reported fatal hospital-acquired MRSA and *Pseudomonas* infections in three out of four patients at the Burns and Plastic Surgery Hospital, in Libya, between August 1999 and August 2002. Since neither rifampicin nor vancomycin was used to treat these patients, their study recommended that vancomycin and rifampicin should be established as the first choice to treat MRSA infection, and infected wounds need uncompromising management with antibiotics prior to skin grafting. A recent a systematic review and meta-

analysis of mupirocin-resistant (MupR) *Staphylococcus aureus* in Africa (Sifaw Ghenghesh et al., 2013), revealed that such strains were indeed reported in Libya (Ahmed et al., 2010; Ahmed et al., 2012). A case study to determine the origin of virulence and multidrug resistance phenotype of a *Klebsiella pneumoniae* isolate from an abdominal wound infection of a patient with a gunshot injury in the thoracoabdominal region concluded that *Klebsiella pneumoniae* ST147 producing OXA-48 and VEB-8 ß-lactamases caused the failure of antibiotic treatment and consequently the death of the patient (Ouertani et al., 2016).

Recently, *Enterobacteriaceae* and Gram-negative rod-shaped nonfermentative bacteria with resistance against third-generation methoxyimino cephalosporins or carbapenems as well as MRSA from war-injured patients from Libya and Syria who were treated at the Bundeswehr hospitals Hamburg and Westerstede, Germany, were assessed by molecular typing, i.e., spa typing for MRSA strains and rep-PCR and next-generation sequencing (NGS) for Gram-negative isolates—the study found that only 7 likely transmission events occurred in the hospitals and the remainder in the country of origin or during the medical evacuation flights (Frickmann et al., 2018). An investigation by spa typing and identification of the Panton-Valentine Leukocidin (PVL) genes of MRSA isolated from clinical sources in Tripoli, Libya, showed that 34% of the isolates were positive for PVL (Ahmed et al., 2017).

An MRSA study incorporating a population of 202 isolates from patients in a Tripoli Medical Centre through the historical period (2008-2014), and characterized by both phenotypic and molecular methods, revealed a diversification of epidemic MRSA strains over time with generally increasing resistance to fluoroquinolone antibiotics (BenDarif et al., 2016). Another study that investigated vancomycin-resistant *S. aureus* (VRSA) in strains isolated from wounds of patients admitted to the Burns and Plastic Surgery Centre in Tripoli, Libya, observed a significant increase in the proportion of MRSA isolates exhibiting higher vancomycin MICs (Zorgani, A. A. et al., 2015).

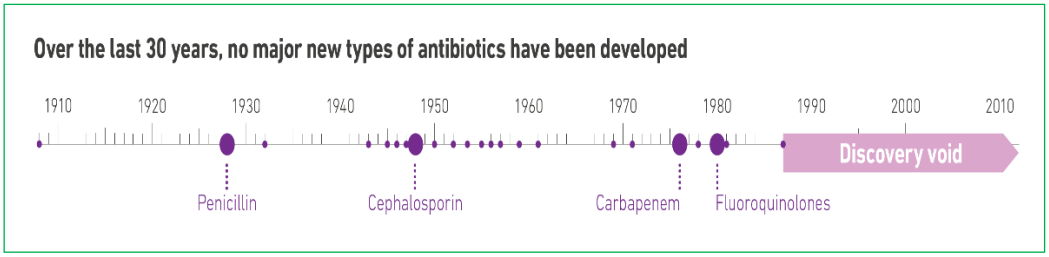
Also, antimicrobial susceptibility patterns of MRSA isolates obtained from health care and community facilities in Libya show a high level of resistance to fusidic acid (Wareg et al., 2014). The same authors also demonstrated prevalence of MRSA was 43%, 37% and 34% in the inpatients (IP-MRSA), outpatients (OP-MRSA) and community carried isolates (CC-MRSA), respectively. Furthermore, in IP-MRSA, all strains were resistant to fusidic acid (100%), none to vancomycin (0%), but to chloramphenicol (31%), gentamicin (37%), erythromycin (48%), streptomycin (56%), cefotaxime (72%), clindamycin (17%) and ciprofloxacin (56%).

The resistance pattern of OP-MRSA strains also revealed that no strains were susceptible to fusidic acid, but resistance to vancomycin was 8%, chloramphenicol 35%, gentamicin 16%, erythromycin 68%, streptomycin 16%, cefotaxime 16%, clindamycin 58% and ciprofloxacin 10%. The sensitivity profile of CC-MRSA exhibited that resistance to fusidic acid was 100%, vancomycin 0%, chloramphenicol 8%, gentamicin 5%, erythromycin 34%, streptomycin 8%, cefotaxime 21%, clindamycin 63% and ciprofloxacin 0%. These results underscore the generally high rates of MDRSA in health care facilities in Libya and the importance of antimicrobial drug surveillance programmes to address this health encumbrance. It is clear that many MRSA strains have developed resistance to most available classes of antibiotics worldwide (Bal et al., 2017; Blair et al., 2015; Chatterjee & Otto, 2013; Guzek et al., 2018; Nagendra Prasad et al., 2019).

1.13 Molecular Mechanisms of Antibiotic Resistance in Multidrug–Resistant *Staphylococcus aureus* (MRSA)

According to the World Health Organization (WHO) Global Report on Antimicrobial Resistance (AMR), antibacterial resistance (ABR) renders infections impossible to control, expanding the risk of the spread of infection to others, prolonging illness and hospitalization, adding to economic and social costs and increasing the risk of death of patients who have become infected with drug-resistant resistant bacteria (Chen et al., 2018; Nelson et al., 2018;

World Health Organization, 2014). Over the last 3 decades, very few major new types of antibiotics with proven efficacy have been developed (Figure 1.4), and without urgent action the world is heading towards a post-antibiotic era, in which common infections and minor injuries can once again become fatal to humans.



Source: World Health Organization. (2014). Antimicrobial Resistance: WHO Global Report. Retrieved from http://www.who.int/drugresistance/documents/surveillancereport/en/, Geneva, Switzerland.

Figure 1.4: Discovery void of antibiotics over the last three decades

Bacterial resistance strategies include reducing entry of antimicrobial agents, expulsion or efflux of antimicrobial agents, inactivation of antimicrobial agents and modification of antimicrobial targets. Bacteria are either intrinsically resistant or acquire resistance to antibiotics via genomic mutations in chromosomal genes and by horizontal gene transfer (Blair et al., 2015). In bacteria, the antibiotic resistance phenotype is encoded by several genes which can transfer between bacteria, enabling the microorganisms to continually mutate and express new resistance mechanisms (Blair et al., 2015; Wright, 2011).

Antibiotic resistance often develops rapidly and spreads at an alarming rate across the globe and among different species of bacteria. Moreover, as a result of sequential, cumulative acquisition of resistance traits against different classes of antibiotics (i.e., cross-resistance), more bacterial pathogens with multiple-drug resistance (MDR) are being reported worldwide (Nagasundaram & Sistla, 2019; Ochotorena et al., 2019; Torre-Cisneros et al., 2018; Upreti et al., 2018). Recent advances have improved our understanding of the molecular mechanisms that underpin antibiotic resistance in bacteria (Foster, 2017).

Intrinsic resistance is naturally encoded by genes and expressed by almost all strains of a particular bacterial species. Many genes are responsible for intrinsic resistance to different classes of antibiotics such as β-lactams, fluoroquinolones and aminoglycosides (Blake & O'neill, 2012; Bush, 1988). Examples of instrinsic resistance is the natural resistance of anaerobes to aminoglycosides and Gram-negative bacteria against vancomycin. Gram-negative bacteria are intrinsically resistant to a wide array of antibiotics because these compounds cannot traverse their outer membrane, i.e., vancomycin inhibits bacterial peptidoglycan cross-linking only in Gram-positive bacteria. Lipopolysaccharides (LPS) present in bacterial cell walls pose another barrier for many antibiotics, but polycationic antibiotics such as gentamicin and colistin, interact with LPS in a process referred to as self-promoted uptake. Efflux pumps of the resistance nodulation cell division (RND) super family play a pivotal role in antibiotic resistance of Gram-negative bacteria (Dolejska et al., 2012).

Bacteria also acquire resistance to antibiotics via mutations in chromosomal genes and by horizontal gene transfer (Admassie, 2018). Since many mechanisms of bacterial resistance to antibiotics have been described in the literature (Abebe et al., 2016; Abraham & Chain, 1940; Admassie, 2018; Alekshun & Levy, 2007; Ashraf et al., 2019; Blair et al., 2015; Brown & Reynolds, 1980; Bush, 1988; Fisher & Mobashery, 2016; Foster, 2017; Jamrozy et al., 2017; Kirby, 1944; Kirmusaolu, 2017; Lowy, 2003; Martins et al., 2013; Munita & Arias, 2016; Pantosti et al., 2007; Peacock & Paterson, 2015; Schito, 2006; Walsh & Wencewicz, 2016; Wright, 2011; Yılmaz & Aslantaş, 2017), not all will be discussed in this thesis. Rather, the few selected mechanisms discussed in the subsections that follow will reflect mainly on methicillin-resistant *Staphylococcus areus* (MRSA) strains since these represent the prototype bacterial species that soon after the clinical use of antibiotics became widespread, were no longer susceptible to penicillin due to the presence of the enzyme, penicillinase, that destroyed the antibiotic (Abraham & Chain, 1940; Brown & Reynolds, 1980; Kirby, 1944; Lowy, 2003).

1.13.1 Horizontal Gene Transfer of Mobile Genetic Elements (MGEs)

Horizontal gene transfer (HGT) of mobile genetic elements (MGEs), including plasmids, transposons and the staphylococcal cassette chromosome (SCC) or by mutations in chromosomal genes that encode virulence and antimicrobial resistance genes mediates the evolution of methicillin-resistant *Staphylococcus aureus* (MRSA), and the emergence of new MRSA clones (Jamrozy et al., 2017). Thus, the pathogenesis of *S. aureus* infection is mediated by several genes that encode virulence factors, e.g., exotoxins—haemolysins and phenol soluble modulins (PSMs)—that disrupt host cell membranes, and immune evasion molecules, e.g., protein A and aureolysin (Foster, 2005; Kirmusaolu, 2017; Vandenesch et al., 2012). The ability of *S. aureus* to thrive as a pathogen is enhanced by rapid HGT. MGEs promote the acquisition of novel virulence factors and are correlated with some of the most potent *S. aureus* virulence molecules, including the pore-formimg Panton-Valentine Leukocidin (PVL) and toxic shock syndrome toxin (Lindsay, 2010, 2014a). MGEs accelerate *S. aureus* adaptation to environmental selection pressures through transfer of antimicrobial resistance genes.

The most clinically significant example of HGT is the Staphylococcal Cassette Chromosome mec (SCCmec) element, which carries the mecA/C gene encoding methicillin resistance (Katayama et al., 2000; Walsh & Wencewicz, 2016). The extensive clinical use of β-lactam antibiotics and ensuing selective pressure for resistance explain the global emergence and spread of SCCmec in *S. aureus*, which has contributed to the evolutionary success of this pathogen in recent years (Chambers & Deleo, 2009; Martins et al., 2013; Poirel et al., 2012; Wright, 2011). This acquired resistance in MRSA is typically conferred by the gaining of a non-native gene encoding a penicillin-binding protein (PBP2a). BPP2a is a homologue of PBP and functions as a bifunctional transglycolylase-transpeptidase: the transglycolylase transfers disaccharide pentapeptide building blocks of peptidoglycan from membrane-bound lipid II to growing polysaccharide chains while the transpeptidase cross-links the glycine cross-bridge of the fourth D-alanine of an adjacent chain (Giesbrecht et al., 1998; Walsh & Wencewicz, 2016).

PBP2a drastically lowers the affinity for β-lactams, thus sustaining cell-wall biosynthesis, the target of β-lactams, even in the presence of toxic inhibitory concentrations of antibiotic (Fisher & Mobashery, 2016; Peacock & Paterson, 2015; Yılmaz & Aslantaş, 2017). PBP2a is encoded by the *mecA* gene, which is carried on a distinct mobile genetic element (SCCmec), the expression of which is regulated by a proteolytic signal transduction pathway comprising a sensor protein (MecR1) and a repressor (MecI). Many of the molecular regulatory role players propelling methicillin resistance mechanisms in *S. aureus* have been delineated (Peacock & Paterson, 2015). Multilocus sequence typing (MLST) has confirmed that MRSA isolates belong to a finite number of clonal complexes (CCs) (Jamrozy et al., 2017), while whole-genome sequencing (WGS) and reconstruction of phylogenetic relationships between MRSA isolates derived from the same CC has demonstrated that MRSA has become widespread predominantly through a process of clonal expansion (Kennedy et al., 2008). Moreover, distinct MRSA clones such as CC5, CC8, CC22, CC30 and CC45 have evolved, like several other MGEs that drive virulence of MRSA (Blair et al., 2015).

1.13.2 Evasion from Innate Immunity

The first line of host protection against invading pathogens is the innate immune system and, to counteract this defence, *Staphylococcus aureus* employ different strategies to evade host defences, including the release virulence factors that impede innate immune defenses (Alonzo & Torres, 2013; Askarian et al., 2018; Banchereau et al., 2012; Bekeredjian-Ding et al., 2017; Brown et al., 2014; den Reijer et al., 2013; Kim et al., 2012; Krishna & Miller, 2012; Peres & Madrenas, 2013; Tkaczyk et al., 2013; Tomar & De, 2013; Wang et al., 2012). Similarly, human host immune systems employ complex mechanisms to identify and subjugate infections (Fink & Campbell, 2018). Phagocytosis and autophagy are two highly conserved processes in macrophages that are crucial to the innate immune response of bacterial infections (Deretic et al., 2013; Zhu et al., 2018). Infection of humans with staphylococcal bacteria triggers a transient increase in anti-staphylococcal antibody (Jacobsson et al., 2010). *Staphylococcus*

aureus infections are typified by the formation of biofilms on medical implants or host tissue which correlate with the persistence of chronic infections. *S. aureus* biofilms have the capacity to evade the macrophage-mediated innate immune responses (Alboslemy et al., 2019). During *S. aureus* infection, the macrophage-mediated innate immune response is stimulated by ligation of pathogen-associated molecular patterns (PAMPs) to pattern recognition receptors (PRRs) (Askarian et al., 2018) expressed on macrophages which, in turn, initiates distinct signaling pathways that converge to activate NF-κB (Johannessen et al., 2013), a chief regulator in the production of numerous cytokines, chemokines and reactive oxygen species to combat *S. aureus*. In spite of this, the extraordinary capacity of *S. aureus* to form biofilms hinders the production of proinflammatory cytokines, and thus destabilizes bactericidal onslaught (Thurlow et al., 2011).

A high initial load of *S. aureus* DNA in blood is associated with sepsis, mortality and persistent immune dysregulation in *S. aureus* bacteraemia patients (Ziegler et al., 2019). Recent studies suggest that *S. aureus* uses a metabolic strategy to overcome last-line antibiotic, daptomycin, and immune attack which encompassed alterations in anionic membrane phospholipid composition induced by point mutations in the phospholipid biosynthesis gene, cls2, encoding cardiolipin synthase. Single cls2 point mutations were sufficient for daptomycin resistance, antibiotic treatment failure and persistent infection.

These phenotypes, mediated by enhanced cardiolipin biosynthesis, led to gain of bacterial membrane cardiolipin function and loss of phosphatidylglycerol, and thus modifications in membrane structure that impaired daptomycin influx and accumulation. The cls2 point mutations also allowed *S. aureus* to evade neutrophil chemotaxis, mediated by the reduction in bacterial membrane phosphatidylglycerol, a previously unidentified bacterial-driven chemoattractant. This mechanism of immune evasion raises hopes for membrane-based therapeutic targeting of *S. aureus* (Jiang et al., 2019).

1.13.3 Resistance to Phagocytosis

The survival of staphylococci within human leukocytes has long been recognized (Graves et al., 2010; Kobayashi et al., 2010; Rogers & Tompsett, 1952). *Staphylococcus aureus* host cell invasion and virulence in sepsis is mediated by multiple fibronectin-binding protein A (FnBPA) repeats (FnBRs) (Edwards et al., 2010). *S. aureus* FnBPA triggers bacterial invasion of endothelial cells via a process that entails fibronectin (Fn) bridging to a5b1 integrins. *S. aureus* SCCmec elements bear major virulence genes, such as the Panton-Valentine leucocidin (PVL) gene (Wu et al., 2019). The PVL-encoding genes, *lukF* and *lukS*, are prevalent among CA-MRSA strains and have previously been associated with the pathogenesis of CA-MRSA infections (Said-Salim et al., 2005). An evaluation of the lysis of human polymorphonuclear leukocytes (PMNs) during phagocytic interaction with PVL-positive and PVL-negative CA-MRSA strains showed no correlation between PVL expression and PMN lysis, suggesting that additional virulence factors facilitate leukotoxicity, MRSA resistance to phagocytosis and, thus, the pathogenesis of CA-MRSA (Said-Salim et al., 2005). *S. aureus* also produces an extensive repertoire of virulence factors, numerous leucocidins and anti-phagocytic factors that inhibit leukocyte adhesion, phagocytosis, sequester host IgG, inhibit complement, inhibits C5a generation, Binds to C5a and IgA and cause phagocyte and leukocyte lysis (Chambers & Deleo, 2009; DeLeo & Chambers, 2009; Ventura et al., 2010).

1.13.4 Lysozyme

Lysozyme plays a significant role in the host's innate immune or constitutive defences against bacterial infection (Flannagan et al., 2015). Lysozyme is a muramidase that cleaves bacterial cell wall peptidoglycan between the glycosidic beta-1,4-linked residues of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) to lyse bacteria (Bera et al., 2005; Wang & Zhu, 2018). Various tissues (mucous membranes, lower respiratory tract or intestinal tract) and body fluids (serum, saliva, sweat and tears) secrete lysozyme and it is also an inducible marker of macrophage and neutrophil activation (Becker et al., 2014; Keshav et al., 1991).

Staphylococcus aureus is higly refractory to lysozyme, which greatly contributes to its persistence (Richards et al., 2015), and success in colonizing the skin and mucosal areas of humans and animals, e.g., the anterior nares in >30% of the human population (Krishna & Miller, 2012; Liu et al., 2018). The precise mechanism of lysozyme resistance in *S. aureus* remains elusive (Bera et al., 2005; Scherr et al., 2013). A Recent study suggests that lysozyme resistance of *S. aureus* blocks IL-1β induction whereas degradation-sentive mutant *S. aureus* induced high levels of IL-1β that mediated bacterial clearance, but at a cost of increased inflammation and necrosis (Bekeredjian-Ding et al., 2014; Shimada et al., 2010). Interestingly, bacteriophage-derived lysins which are cell-wall-hydrolytic enzymes represent a feasible novel class of antibacterial strategies to improve clinical outcomes of serious antibiotic-resistant staphylococcal infections (Indiani et al., 2019).

1.13.5 Host-Pathogen Interaction of *Staphylococcus aureus*

The skin and mucosa of humans are often colonized by *S. aureus*. The sources of staphylococcal infection have their origin basically in patients or hospital personnel, although surfaces contaminated by mites and medical devices do play a significant role as intermediate sources. According to estimates in the USA, 30% of healthy individuals are colonised by *S. aureus* in the skin or mucosa (Gorwitz et al., 2008). MRSA can be available as a tribute agent increasing antibiotic resistance in human contagion due to their presence in wastewater , including antibiotic-resistant bacteria, antibiotic resistance genes (Pruden, 2014). Another study in South China was still limited to *S. aureus* in nasal passages which is the main source of the transportation (Chen, B. J. et al., 2017).

An important mechanism for promoting colonization is the adherence of *S. aureus* cell wall anchored (CWA) proteins to surface components; some of these components are fibrinogen, fibronectin and cytokeratins of either nasal epithelium or epidermal keratinocytes. By recognising adhesive matrix molecules, CWA proteins interact with microbial surface components. These matrix molecules (MSCRAMMs) include Fnbp A and Fnbp B, fibrinogen

binding proteins (CIfA and CIIB), iron regulated determinants IsdA and wall teichoic acid WTA (Borbone et al., 2008). MRSA ST398 isolates were obtained through the quarantine period in Japan from imported swine against antimicrobial resistance, generating feedback when monitoring imported animals would be important (Furuno et al., 2018). Gram-positive organisms, such as *S. aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis* have dynamic cell envelopes with peptidoglycan (PG), which is a target for antibiotics, teichoic acids (TAs), capsular polysaccharides (CPS), surface proteins, and phospholipids. These components can undergo modification to promote pathogenesis (Rajagopal & Walker, 2015).

1.14 Problem Statement

Globally, the ever-increasing prevalence of hospital-acquired infections (HAI), particularly nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA), is an emergent public health concern (Denkinger et al., 2013; Rivero-Perez et al., 2012; Schweickert et al., 2011; Thurlow et al., 2012; van Duijn et al., 2011). Molecular characterization and typing of MRSA have become indispensable diagnostic tools for efficient infectious disease surveillance systems, to delineate its epidemiological trends and to drive effective infection control strategies (Bettin et al., 2012; Durlach et al., 2012; Gowrishankar et al., 2013; Iwao et al., 2012; Joo et al., 2012; Kaier, 2012; Kawaguchiya et al., 2013; Lin et al., 2012).

Moreover, MRSA is endemic in many hospitals and presents a considerable socio-economic burden worldwide, especially in developing countries where clinical care is often significantly compromised (Buzaid et al., 2011; Chamchod & Ruan, 2012; Menif et al., 2011; Moremi et al., 2012; Schaumburg et al., 2011; Shittu et al., 2011; Shrestha et al., 2010; Song et al., 2011). MRSA prevalence is considered a major obstacle to effective antimicrobial therapy in hospitals in Libya, and there is a dire need to establish a comprehensive surveillance and prevention programme in the country to reduce MRSA and other antimicrobial-resistant pathogens in Libyan hospitals (Buzaid et al., 2011). This study aims to identify antibiotic susceptibilities that specifically target MRSA strains isolated from stored patient samples in selected hospitals

and laboratories in Libya. In addition, an attempt was made to analyze the extent of MRSA contamination of poultry samples obtained from various locations in the country.

1.15 Aim of the Study

The aim of this study was to compare antibiotic sensitivities of *Staphylococcus aureus* isolates in human patient samples from Misurata hospitals and laboratories as well as poultry samples from commercial markets in Libya.

1.16 Objectives of the Study

The objectives of the study were to:

1. Analyze laboratory and hospital samples from patients as well as poultry samples for bacterial and fungal growth, using the blood agar test, Gram-stain test, mannitol salt agar test, the catalase test, the coagulase test, antibiotic susceptibility tests, the Mueller-Hinton agar test, minimum inhibitory concentration (MIC) tests and the Polymerase Chain Reaction (PCR).

2. Compare traditional bacterial culture methods that are used to measure *Staphylococcus aureus* methicillin-resistant (MRSA) strains with modern molecular methods to isolate the *mecA1* and *mecA2* genes, using PCR.

3. Determine the diagnostic profile of the bacterial and fungal species in patient and poultry specimens.

4. Evaluate the *Staphylococcus aureus* antibiotic sensitivity and resistance profiles for patient and poultry samples.

5. Compare *Staphylococcus aureus* antibiotic sensitivity and resistance profiles in patient specimens according to gender, age group and location (site collected).

6. Compare *Staphylococcus aureus* antibiotic sensitivity and resistance profiles in poultry samples according to location and different parts of the chicken after slaughter.

7. Identify and detect the MRSA contamination in chicken samples.

1.17 Research Question

This study was guided by the following research question:

Are Staphylococcus aureus isolates from poultry sourced from commercial markets and human isolates from patient samples in selected hospitals and laboratories in Libya resistant to most antibiotics currently indicated for control and elimination of the microorganism?

CHAPTER 2

RESEARCH METHODOLOGY

2.1 *Staphylococcus aureus* Growth Conditions

Bacterial isolates from patient swab samples were obtained from the hospitals and laboratory as well as poultry (chicken) samples were cultured in petridishes at 37ºC for 16-18 hours, using blood agar, mannitol salt agar, MacConkey agar, tryptic soy agar / Trypticase™ (TSA) and test chemicals to identify and compare important bacteria and recognize their isolates (Mary Jo Zimbro et al., 2009; Sneath et al., 1986). A single colony forming unit (CFU) was inoculated into tryptone soy broth (TSB) and incubated for 5-8 hours.

2.2 Sample Collection

Strains of *Staphylococcus aureus* were collected in one year between 1 January 2014 and 31 December 2014. Microbiological tests were done in three hospitals and one private Central Laboratory in Misurata (Table 2.1), and 5 poultry stations, in order to study the problem, to improve the diagnosis of *Staphylococcus aureus* and how the bacteria can be spread from poultry to humans in the city of Misurata, Libya. Samples from human and poultry were collected (n=962), with 657 samples from humans and 434 from poultry.

These samples were collected from blood, urine, stool, ear swabs, spinal cord fluid (CSF), wounds, and sputum. Other samples were collected from the intensive care unit (ICU) and from the medical analysis departments at the two main central hospitals (Central Hospital and National Cancer Institute, Assawa International Hospital in Misurata and the Central Laboratory in Misurata). In addition, poultry swab samples were also collected in sterile containers from different locations, namely, Abdurrahman Abahy, Alfetory, Baser and

Alkherobp Market in Misurata. All samples were immediately placed in larger containers filled with ice and then transferred directly to the laboratory where they were analyzed and diagnosed.

Table 2.1: Institutions where samples were collected

Institution	Section
Central Hospital of Misurata	Inpatients Wards
	Intensive Care Unit
	Outpatients Department
	Chemistry and Haematology Laboratories
Assafwa International Hospital	Outpatients Department
National Cancer Institute	Surgery Department
	Outpatients Department
Central Laboratory Misurata	Outpatients Department

- Clinical Sample

Samples of blood, urine, sputum, spinal cord fluid, semen, faeces, swabs, body post-operative smears, throat swabs and wound swabs were obtained from patients of four health care institutions, namely, the Central Hospital, Al Safwa International Hospital, National Cancer Institute and Central Laboratory Misurata.

2.3 Culture, Media and Staining

2.3.1 Blood Agar

Blood agar was prepared by dissolving 10 g Lab-Lemco powder, 5 g sodium chloride (NaCl), 10 g peptone neutralized and 15 g in 1 litre of water and then neutralized to pH 7.3. The method of preparation of blood agar was as follows: 40 g of powder was weighed, dissolved and sterilized by autoclaving for 15 minutes at 121°C and pressure 15 psi. After the solution was cooled to 50°C, 6% sterile defibrinated blood was added. The solution was poured into a petri-dish to a height of approximately 2 mm.

Finally, the media were stored in cool dry place (refrigerated 4-8°C). Haemolysis is the breakdown of red blood cells (Figure 2.1). The colonies of bacteria *Staphylococcus aureus* are used to induce haemolysis when grown on blood agar after 24 hours incubation at 37°C (Mary Jo Zimbro et al., 2009; Sneath et al., 1986). Blood agar is also used to classify certain microorganisms and is particularly useful in classifying streptococcal species. If there are transparent areas around the bacterial colonies after 24 hours of incubation at 37°C then the test is considered as a positive result and when there is no transparent zone visible around the bacterial colonies, then the test result is negative.

Figure 2.1: *Staphylococcus aureus*-induced haemolysis in blood agar culture

2.3.2 Inorganic Chemicals

In this study, all biochemical and inorganic substances referred to in this chapter were obtained from the following companies: Oxoide, London in the United Kingdom, Sweden and Italy. Distilled and sterilized water were used in all preparations. Not all media were prepared in the laboratory - this depended on the manufacturers' instructions for the use of nutrient agar (Oxoid UK), and tryptone soy broth (Oxoid UK).

2.3.3 Gram-Stain

The stains used were crystal violet, Gram's iodin, acetone alcohol, safranin counterstain. All samples were tested with a Gram-stain to identify the bacteria *S. aureus* using a clean and sterilized microscope slide, and then passed through a flame to remove any residual fat. A drop of distilled water was put on the slide by sterile loop. A single colony from a pure culture of *S. aureus* was then mixed thoroughly with the water on the slide and spread evenly. The slide was allowed to air dry in a dust-free environment.

For the smear not to be washed off during the staining, it was fixed on the slide by passing the slide, right side up, over a flame three or four times. After fixation, the smear was flooded with crystal violet solution and allowed to stand for one minute and then the smear was washed gently with tap water. Then, the smear was flooded with Gram's iodine. The solution was allowed to stand for one minute. As a next step, the smear was washed with tap water slowly until the excess iodine solution was removed and then the smear was decolorised. Step three was the addition of acetone alcohol which usually takes 5 to 10 seconds. After that, the smear was slowly washed with tap water.

In step four, the slide was flooded with safranin counterstain for 30 to 60 seconds and again washed gently with tap water, followed by blot drying. Finally, the stained smear was examined by the 100× (oil immersion) of the microscope (OptikA). Upon examining the slides by microscope, the cells were differentiated by stain colour. Gram-positive bacteria (Gram+) have a dark blue, while Gram-negative bacteria (Gram–) have a pink to red colour. In our case, we found that our bacteria, which is *S. aureus*, showed a dark blue colour, meaning that it is a Gram-positive bacterium.

2.3.4 Nutrient Agar Media

Nutrient agar is prepared aseptically by dissolving 1 g Lab-Lemco powder, 2 g yeast extract, 5 g peptone, 5 g sodium chloride and 15 g and agar into one litre of distilled water, and the pH

adjusted to 7.4 at 25°C. The solution in a closed sterile bottle was mixed and sterilized by autoclaving for 15 minutes at 121°C and 15 psi. After this, the solution was mixed and then poured into petri dishes. Finally, the media was stored in a cool place.

2.3.5 Nutrient Broth

Nutrient broth was prepared according to instructions (Oxoide, England) by dissolving15 g of nutrient broth powder in one litre of distilled water. After mixing well and distributing into final containers (test tubes), the solution was sterilized by at 121°C for 15 minutes and 15 psi. After that, it was kept tightly closed, away from bright light in a cool dry place (refrigerator 4-8°C). It was used to activate the bacteria.

2.3.6 Mannitol Salt Agar

All samples were tested with mannitol salt agar (Oxoid, England) to test Gram-positive bacteria to compare between *Staphylococcus aureus*, and Staphylococci coagulase-negative bacteria. *Staphylococcus aureus* is able to ferment on the mannitol salt agar and gives a yellow colour, and Staphylococcus coagulase-negative has a red colour as shown in Figure 2.2. The mannitol salt agar contains a height ratio of salt (7.5% NaCl) which stops the growth of many bacteria other than *Staphylococcus aureus*.

The agar contains the sugar mannitol, which when fermented by the activity of *Staphylococcus aureus* yields acidic product(s), so changing the value of the fundamental pH of the culture media. The phenol red indicator exhibits a yellow colour in acidic solutions and otherwise a reddish colour. When it shows a yellow colour in the results, it is called *Staphylococcus aureus*. Mannitol salt agar (MSA) contains powder of mannitol salt agar (MSA), Lab-Lemco powder,1.0 g, peptone, 10.0 g, mannitol, 10.0g, sodium chloride, 75.0g, phenol red, 0.025 g and agar 15.0 g in one litre of distilled water and then the pH of the solution is adjusted to 7.5 0.2 at 25°C.

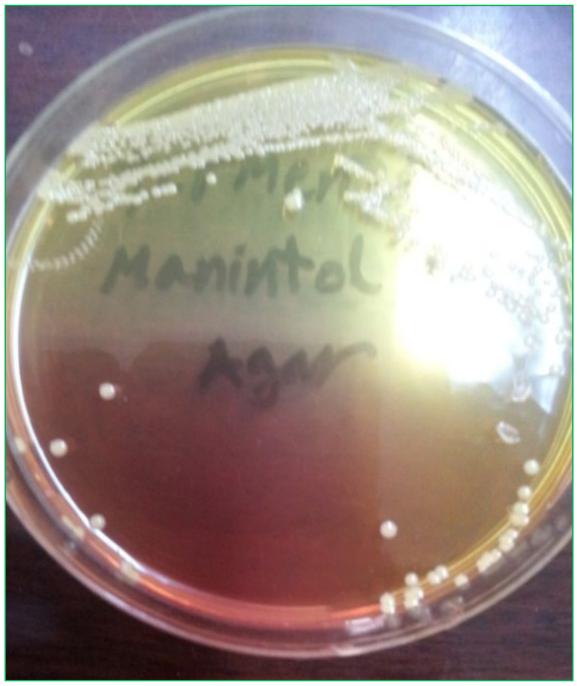


Figure 2.2: Growth of *Staphylococcus aureus* and its ability to ferment sugar mannitol

2.3.7 MacConkey Agar

MacConkey agar was prepared according to instructions (Oxoid, England). It is formulated to be a selective medium used for the isolation and differentiation of negative Gram bacteria rods, particularly members of the family *Enterobacteriaceae* and the genus *Pseudomonas* (De la Maza et al., 2013; MacConkey, 1905; MacConkey, 1908; Ryan & Ray, 2004). MacConkey agar contains 17 g peptone (pancreatic digest of gelatin), 3 g protease peptone (meat and casein), 3 g lactose monohydrate, 10 g bile salts and 1.5 g MacConkey agar prepared by weighing 49.53 g of dehydrated medium into 1000 ml purified distilled water.

The solution was dissolved completely in one litre of distilled water in a closed sterile bottle. Then the solution was mixed and sterilized by autoclaving for 15 minutes at 121°C and 15 psi, after which the solution was mixed well and cooled to 45-50°C before being poured into petri dishes. Finally, the media was stored in a cool dry place (refrigerator). Lactose-fermenting bacteria strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red colour is due to the production of acid from lactose, absorption of neutral red and a

subsequent colour change of the dye when the pH of the medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella*, are colourless and transparent and typically do not alter the appearance of the medium. Also, *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature. The reactions of various species to MacConkey agar are summarised in Table 2.2.

Table 2.2: Reactions of various species to MacConkey agar

Organism	Colour	Remarks
Escherichia coli	Red/Pink	Non-mucoid
Aerobacter aerogenes	Pink	Mucoid
Enterococcus species	Red	Minute, Round
Staphylococcus species	Pale Pink	Opaque
Pseudomonas aeruginosa	Green-Brown	Fluorescent Growth
(MacConkey, 1905; MacConkey, 1908; Ryan & Ray, 2004; Maza et al., 2013)		

2.3.8 The Catalase Reaction

This test is used to compare between *Staphylococcus* and *Streptococcus*; all of these species are Gram+ cocci bacteria. A catalase test gives a positive result with *Staphylococcus* and a negative result with *Streptococcus*. Catalase is an enzyme present in most cytochrome-containing aerobic and facultative anaerobic bacteria. An important exception is the *Streptococcus* species. The test is performed by exposing the test organism to hydrogen peroxide and observing the immediate oxygene production. Reagents and equipment used in the test are 1) Hydrogen peroxide (3% H_2O_2), normally stored at 15-30°C, 2) glass slides and 3) Sterile sticks or inoculating loops are used. The test is carried out by transferring a single bacteria colony (pure culture) from a petri dish to a clean microscope slide and adding a drop of solution at a concentration of 3% H_2O_2.

❖ Reagents and Equipment

1. Hydrogen peroxide (3%), stored at 15-30°C

2. Slides
3. Sterile sticks or inoculating loop

❖ Procedure

Using a loop, take an 18-24-hour pure culture and place it on a clean microscope slide. Using a Pasteur pipette or a dropper, add a drop of 3% H_2O_2. We placed a single colony of the bacteria on the slide and added one drop of H_2O_2 and observed immediate oxygen bubbling. This means that our bacteria were *Staphylococcus* and not *Streptococcus*, because *Streptococcus* bacteria do not interact with hydrogen peroxide to produce immediate oxygen bubbling.

2.3.9 Coagulase Test

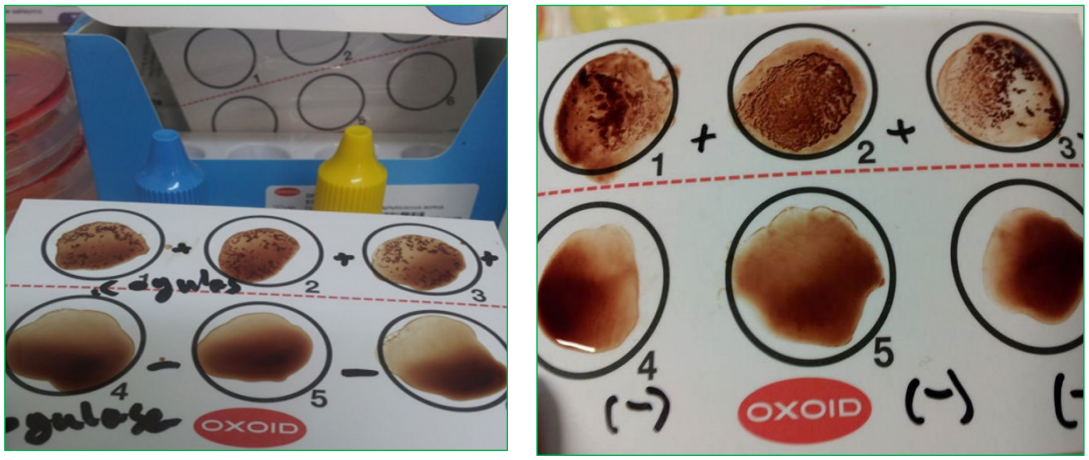
We used a coagulase test in clinical microbiology laboratories, according to instructions (Oxoid, UK) to compare between bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*. When the *S. aureus* are positive for a coagulase test, an enzyme that induces plasma coagulation by activating prothrombin produces a positive coagulase test, where *Staphylococcus epidermidis* is negative for the coagulase test. The coagulase test was done by two methods—slide method and tube method.

2.3.9.1 Coagulase Test (Slide Method)

There are two kinds of coagulase enzyme, bound coagulase enzyme and free.

❖ Procedure

One drop of rabbit plasma kit (Oxoid, UK) with a single colony was placed inside a small circle on a clean glass slide; after that, a single colony was added by using a wooden pick or sterile loop and mixed well to comparatively control coagulase-positive and coagulase-negative tests. In the plasma, fibrin threads form between the cells due to coagulase, causing them to agglutinate. Thus, there is a visible clumping of cells within 10-15 seconds. This test was done for the bound coagulase enzyme as shown in Figure 2.3.



1, 2 and 3: if it is a positive result, there will be visible clumping of cells within 10-15 seconds. This test was done for the bound coagulase enzyme; **4, 5 and 6:** without agglutination, it shows negative results.

Figure 2.3: *Staphylococcus aureus* coagulase test (slide method)

2.3.9.2 Coagulase Test (Tube Method)

All isolated *S. aureus* strains were used to confirm the diagnosis; the test was used for the free coagulase.

❖ Procedure

Three test tubes were taken and labelled 'test', 'negative control' and 'positive control'. Every tube was filled with one ml of a 1-in-10 diluted plasma solution. To the tube labeled 'positive control', 0.2 ml of overnight broth culture of known *S. aureus* was added; to the tube labelled 'negative control', 0.2 ml of sterile broth was added. The tubes were incubated in a Schwabach (Germany) incubator at 37°C and the suspensions were checked at half hourly intervals for a period of four hours. The positive result is shown by agglutination or clotting of the plasma, which remains in place even after inverting the tube. If the test is negative until four hours at 37°C, the tube is kept at room temperature for overnight incubation. If a clot is observed due to the reaction of coagulase enzyme with the rabbit plasma within four hours at 37°C or more overnight incubation at 37°C, the result is considered as Coag+, but if there is no glutination or clotting, the result is considered as Coag-; if the formation is observed after 24 hours, the result is considered as Coag– (Koneman et al., 1997).

2.3.10 Antibiotic Susceptibility Test

In vitro susceptibility tests were performed on Mueller-Hinton agar by two methods:

1. The disk diffusion method, as described by the Clinical and Laboratory Standards Institute (CLSI) standards.

2. The Minimum Inhibitory Concentration (MIC) as described by the Etest

❖ Method, in Accordance with CLSI Standards

All MRSA strains were determined by disk diffusion method and confirmed by MIC method using the Oxacillin Etest (Biodisk, Solna, Sweden).

2.3.11 Mueller-Hinton Agar

Mueller-Hinton agar (Oxoid.UK) has the following composition g/l: beef dehydrated infusion (300), casein hydrolysate (17.5), starch (1.5), agar (17), pH7.3 ±0.1 at 25°C. This is prepared in media, according to the manufacturer's instructions (Oxide UK). Mueller-Hinton agar was prepared by weighing and dissolving 38 g in one litre of distilled water and boiling it to dissolve it fully. Then, the solution was sterilized by autoclaving at 121°C for 15 minutes and placed in petri-dishes. Finally, the media was stored refrigerated between 4-8°C. In vitro susceptibility tests were performed on Mueller-Hinton agar by the two methods referred to above.

2.3.11.1 Minimum Inhibitory Concentration (MIC)

Strips were removed from the refrigerator and allowed to reach room temperature (+4° C / approx. 30 minutes, -20°C / approx. 60 minutes) until the packages reached room temperature; the strips were used immediately. We ensured wet condensation on evaporation of the outer surface of the package completely before opening. The Mueller-Hilton agar is used for checking the reliability of MIC tests using oxacillin for detecting sensitive- and resistant-methicillin-resistant *S. aureus* (MRSA) (Mary Jo Zimbro et al., 2009; Thornsberry & Mcdougal, 1983).

In this study, we used the BSAC methodology described by Howe & Andrews (2012). We used oxacillin in this study for *Staphylococci*. Sensitivity suggests bacteria, I and R sensitivity point to bacterial resistance. M and Z point to the MIC stop and zone respectively. DCon is the disk content in µg/volume. BSAC is suggested by the British Society of Antimicrobial Chemotherapy. Pure single colonies of *Staphylococcus aureus* from blood agar, after it is diagnosed and then vaccinated in the nutrient broth for bacteria, were activated by a sterile cotton swab from the suspension, and the fluid removed by pressing on the fluid. The content fluid of MRSA is grown on Mueller-Hilton agar by sweeping of the surface evenly in all directions from the same swab. After that, the oxacillin strip is placed by sterile forceps on the middle surface and left on the agar surface for 15 minutes at the implant table or in an incubator to dry and an oxacillin strip is placed on the medal of the Mueller-Hilton agar.

The dishes were incubated at 37°C for 24 hours for MRSA and incubated for 48 hours if the result is negative after 24 hours. Biodisk (Solna, Sweden) is the most commonly used antimicrobial method. It can be determined by reading the meter on the strip at the intersection of bacterial growth (Reller et al., 2009). Electronic testing is cost-effective, flexible and easy, even in smaller laboratories. *Staphylococcus aureus* on the Mueller Hinton agar is resistant to oxacillin at concentrations from 0.015 µg/l, 0.03 µg/l, 0.06 µg/l, 0.12 µg/l, 0.25 µg/l, 0.5 µg/l and 1 µg/l and sensitive to the antibiotic from 2 µg/l, 4 µg/l, 8 µg/l ,16 µg/l, 32 µg/l, 64 µg/l, 128 µg/l, 256 µg/l and oxacillin. The strips were used immediately as soon as the packages' temperature reached room temperature. A summary of the minimum inhibitory concentrations (MIC) and the zone breakpoints, is based on the data of Howe & Andrews (2012).

- Reading Plates

The petri dishes were read only when sufficient growth was observed after 24 hours for MRSA and 48 hours for MRSA. Besides, MIC was read where the oval scale intersects the MIC measurement on the strip.

2.3.11.2 Disk Diffusion Method

The Minimum Inhibitory Concentration (MIC) was described by the E-test method in accordance with CLSI standards. All MRSA strains were determined by disk diffusion method and confirmed by MIC method using the Oxacillin Etest (AB Biodisk, Solna, Sweden, 1999).

❖ Procedure

Sensitivity tests were conducted using standard saturated antibiotic Kirby-Bauer disc diffusion as described by Bauer et al. (Bauer et al., 1966). *S. aureus* colonies were isolated from the blood agar plates (Oxide, UK). Of the colonies, 4-5 pure ones were transferred for examination of growth from the original dish after diagnosis. *S. aureus* was added to a test tube containing tryptone soy broth and placed in the incubator for 24 hours under temperature 37±2°C to activate the bacteria. After adjusting the turbidity meter, the inoculation was started.

The meter was set to 0.5 McFarland standards by spectrophotometer; dishes containing Mueller-Hinton agar were created by dipping cotton swabs into the tube containing the bacteria colony and wiping the surface of the dish with the cotton swab to induce growth spreads evenly. A waiting period of 3-5 minutes ensued to dry the surface and then distributed discs selected by placing four tablets of antibiotics handled with sterile forceps in each dish so that they were consistent and a sufficient distance for inducing the reaction was allowed. It was ensured that there was no overlap between antibiotic tablets. The dishes were then placed in the incubator for 18-24 hours at a temperature of 37.2°C and then the inhibitory region was measured with a ruler and compared to the standard tables as shown in Table 2.3.

2.4 Bacterial Strains and Growth Conditions

The bacterial isolates used for this analysis were MRSA. One strain was obtained from the Misurata City Culture Collection. The bacterial isolates were cultured on tryptone soy agar (TSA) (Oxide, UK) plates and incubated using Schwabach (Germany) incubator TYP:BM400 at 37ºC, for 16-18 hours.

Table 2.3: Minimum inhibitory concentrations (MIC) and zone breakpoints

Class	Antibiotic	R_M>	I_M	S_M	D_{cont}	R_Z	I_Z	S_Z
Aminoglycosides	Amikacin A	16	8–16	4	30	18	–	19
Macrolides	Erythromycin	0.5	–	0.5	5	19	–	20
Aminoglycosides	Gentamicin	1.0	–	1	10	19	–	20
Aminoglycosides	Kanamycin	–	–	–	–	–	–	–
Penicillases	Oxacillin	2.0	–	2	1	14	–	15
Aminoglycosides	Streptomycin	–	–	–	–	–	–	–
Tretracyclines	Tetracycline	1.0	–	1	10	19	–	20
Aminoglycosides	Vancomycin	4.0	–	4	5	11	–	12

Summary of the minimum inhibitory concentrations (MIC) and the zone breakpoints, based on the data of Andrews and Howe (2011) and Howe and Andrews (2012) in the BSAC methodology, of the antibiotics, used in this study, for Staphalococci. R indicates the resistance, I, the intermediate sensitivity and S the sensitivity. The subscripts M and Z represent the MIC and zone breakpoints, respectively. DCon is the disc content in micrograms/volume. BSAC denotes the British Society for Antimicrobial Chemotherapy. – indicates that data were not available. **Notes**-Choice of antibiotic oxacillin instead of methicillin because it is sturdier when tested in the laboratory (Blomquist, 2006).

A single colony-forming unit (CFU) was inoculated into tryptone soy broth (TSB) (Oxoid, UK) and incubated for 5-8 hours, with shaking, at 37°C to a turbidity equivalent to 0.5 McFarland standard (~1×10^8 to)2×10^8 CFU/ml).

2.5 Polymerase Chain Reaction (PCR)

Two methods were used for the PCR-Techne-Tc-412.model FTC41s5d. Each method was repeated three times.

2.5.1 DNA Extraction and Purification PCR

PCR analysis was performed in triplicate on all samples. For DNA extraction, 1.5 ml cell suspension was grown overnight at 37ºC in TSB. It was then centrifuged at maximum for 5 minutes. The supernatant was removed and the pellet kept. The pellet was then resuspended in 200 ml lysis buffer and incubated at 37ºC for 15 minutes then at 100ºC for 10 minutes. The supernatant was removed and the pellet stored at -20ºC for future use. The lysis buffer contained 1% Triton X-100, 0.5% Tween-20, 1 mM Tris-HCl (pH 8.0), 1 mM EDTA. T25 l

PCR volume 200 M dNTP S, 1.5 taq, 0.2 M of each primer sterile distiller water to 24 l, 1 l Template DNA.

2.5.2 Cryostorage of the Samples

The microorganisms were stored at depressed temperatures using a mechanical technique called microbank that displays the lower possibility of the disorder and permits ready access to stored material. Microbank is a sterile vial containing porous beads which serve as transport to support microorganisms.

2.5.3 Polymerase Chain Reaction (PCR)

2.5.3.1 Primers

The following primers that produced 533 bp fragment were used:

mecA1 AAAATCGATGGTAAAGGTTGGC

mecA2 AGTTCTGCAGTACCGCATTTG

2.5.3.2 Cell Lysis, Bacterial DNA Extraction and Purification

In this procedure, 0.5 µl of distilled water was added to each tube containing a colony of MRSA and then vortexed well and put in a centrifuge for five minutes before carefully removing the supernatant. The pellet was resuspended in 200 µl TE buffer and vortexed and centrifuged for five minutes and 400 µl Digestion solution in 200 µl sample added from step one and vortexed, then 3 ml of proteinase K solution (2 mg/150 µl) was added to the sample and incubated at 55°C in a waterbath for five minutes; after that, 260 µl of 100% ethanol was added and mixed well and applied to all samples in new tubes with filters, centrifuged at 8.000 x g (10,000 rpm) for two minutes. Then the supernatant was removed; the flowthrough was discarded in the collection tube and 500 µl of wash solution was added and spun as above. The flowthrough in the collection tube was discarded, 500 µl of wash solution was added and spun again. The flow through was discarded for an additional minute to remove the residual amount of wash solution

from the EZ-10 Column after adding 30-50 µl Elution Buffer (EB) into the centre part of membrane in the Column. A clean 1.5 ml Eppendorf tube was incubated and reverse transciptase (RT) was incubated for two- three minutes and additionally, incubation at 37°C or 50°C for two minutes to increase recovery or yield. Again, the sample was spun at 8.000 x g for two minutes to elute DNA from the column. The pellet was taken and 20 µl EB added and mixed in the primary tube for lengthy storage, and the liquid kept at-20°C for genomic DNA analysis.

2.5.3.3 Confirmation of Identification by Polymerase Chain Reaction

PCR is perfect to magnify the 553 bp portion of the mecA (resistant) of *S. aureus*. The PCR reaction mixture included 0. 5 mM KAPA DNTP mix, 2.5 mM $MgCl_2$, 2.5X reaction buffer, 0.5 U/ml Taq DNA polymerase, 5 µl DNA and 0. 4 µM of every primer, with added PCR grade water, in a total volume of 25 µl. The PCR situation was as follows: first denaturation at 94°C for five minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds and annealing at 55°C for 30 seconds and extension to 72°C for one minute. A final extension was applied at 72°C for five minutes, using Mi-Bacterial Genomic DNA Isolation Kit (Murakami et al., 1991). A representative gel of PCR results of *Staphylococcus aureus* samples showing *mecA1* and *mecA2* is shown in Figure 2.4.

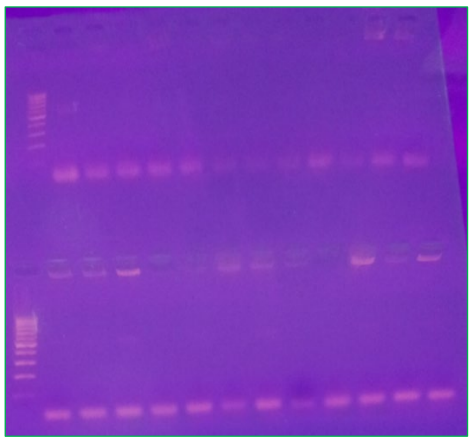


Figure 2.4: Representative PCR assay profile of *S. aureus* samples showing *mecA1* and *mecA2* genes

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Analysis of Laboratory and Hospital Samples

3.1.1 Introduction

In this study, 657 laboratory and hospital samples from patients were collected. Patient samples collected were from blood culture, cerebrospinal fluid (CSF), eye swab, lung swab, semen, sputum, stool, swabs, swabs from operation, throat swabs, urine and wound swabs. The samples were taken at the intensive care units (ICUs), laboratories and surgical departments of the three main hospitals, as well as at a private clinical laboratory, all located in Misurata, Libya. These institutions are the National Cancer Institute Misurata (NCIM), the Central Hospital Misurata (CHM) the laboratory of the Al Saffwa International Hospital (AIH), and the Misurata Central Laboratory (MSL).

Of the total number of 657 samples, 84 were found to be coagulase-positive, mannitol salt agar (MSA) positive (blood haemolytic positive), catalase-positive and Gram-stain positive *Staphylococcus aureus* (*S. aureus*); this amounted to 73 *S. aureus* which tested resistant and sensitive to the antibiotic oxacillin, as established by the disc sensitivity test and the polymerase chain reaction (PCR) test. From the CHM, 378 (58%) samples were isolated and tested; of these; of the total of 36 samples 23 were found to be methicillin-resistant *S. aureus* (MRSA), while 22 (61.1%) were *S. aureus* positive PCR (MRSA) and sensitive to oxacillin by the disc test, and 1 (2.8%) of samples were PCR negative. Furthermore, 110 (17%) samples were isolated and tested from the AIH; of these samples, 10 were MRSA and sensitive to oxacillin by the disc test, and 10 (27.8%) samples were PCR-positive. Also, 41 (6%) of the samples were isolated and tested from the MCL; of these 1 was MRSA and sensitive to

oxacillin by the disc test, and 1 (2.8%) was PCR-negative. Finally, 128 (19%) samples from the NCIM were isolated and tested; of which 2 were MRSA and sensitive to oxacillin by the disc test of the total of 36 PCR (MRSA) samples, and 2 (5.6%) were PCR-positive.

3.1.2 Patient Samples Collected and Tested

Table 3.1 and Figure 3.1 show a comparison between the number (n) and percentage (%) distribution of patient samples collected and tested from the various institutions. The majority of the samples (n=378; 58%) were collected from Central Hospital Misurata (CHM), followed by the National Cancer Institute of Misurata (NCIM, n=128; 19%), Assafwa International Hospital (AIH, n=110; 17%) and Misurata Central Laboratory (MCL, n=41; 6%).

Table 3.1: Patient samples collected and tested from the various institutions

Institution	Number (n)	Percentage (%)
AIH=Assafwa International Hospital	110	17%
CHM=Central Hospital Misurata	378	58%
MCL=Misurata Central Laboratory	41	6%
NCIM=National Cancer Institute Misurata	128	19%
Total	**657**	**100%**

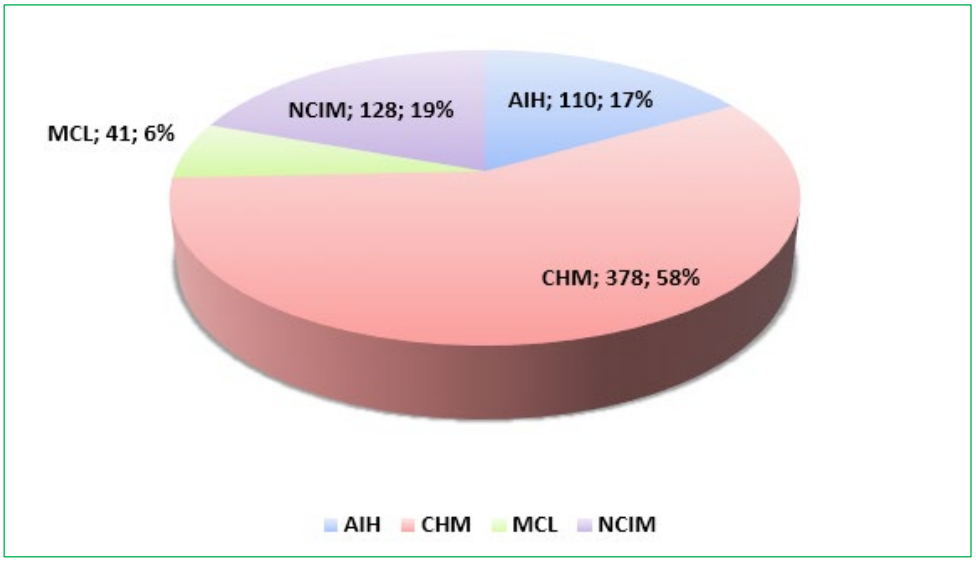


Figure 3.1: Percentage distribution of samples collected from patients at the various institutions

Table 3.2 and Figure 3.2 show a comparison between the number of samples collected and tested from patients, as a percentage of the total, at the various institutions and their departments. The highest number of specimens was collected from the CHM (n=335; 51%), followed by the AIH (n=110; 16.74%). The lowest number of samples was collected from the NCIMDS (n=22; 3.35%), with the number from MCL (n=41; 6.24%) being the second lowest.

Table 3.2: Patient samples collected and tested from the various institutions and their departments

Institution	Number (n)	Percentage (%)
AIH	110	16.74%
CHM	335	50.99%
CHMICU	43	6.54%
NCIM	64	9.74%
NCIMDS	22	3.35%
NCIMICU	42	6.39%
MCL	41	6.24%
Total (n)	**657**	**100%**

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; NCIMDS=National Cancer Institute Misurata Department of Surgery; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata; NCIMICU=National Cancer Institute Misurata Intensive Care Unit

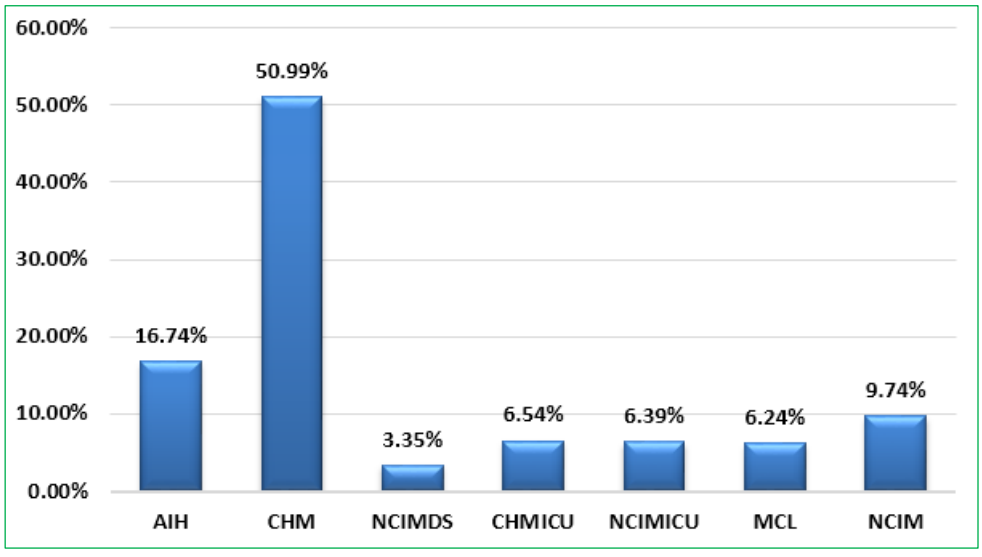


Figure 3.2: Samples collected and tested from patients at various institutions and their departments

Table 3.3 and Figure 3.3 show the breakdown of the number of patient specimens emanating from hospitals and laboratories. The highest number of specimens was collected from hospital patients (n=449; 68%) of the total specimens (n=657), while the lowest number collected was from the laboratories (n=208; 32%).

Table 3.3: Patient specimens emanating from hospitals and laboratories

Source of sample	Frequency	Percent
Laboratory	208	32%
Patient	449	68%
Total	**657**	**100%**

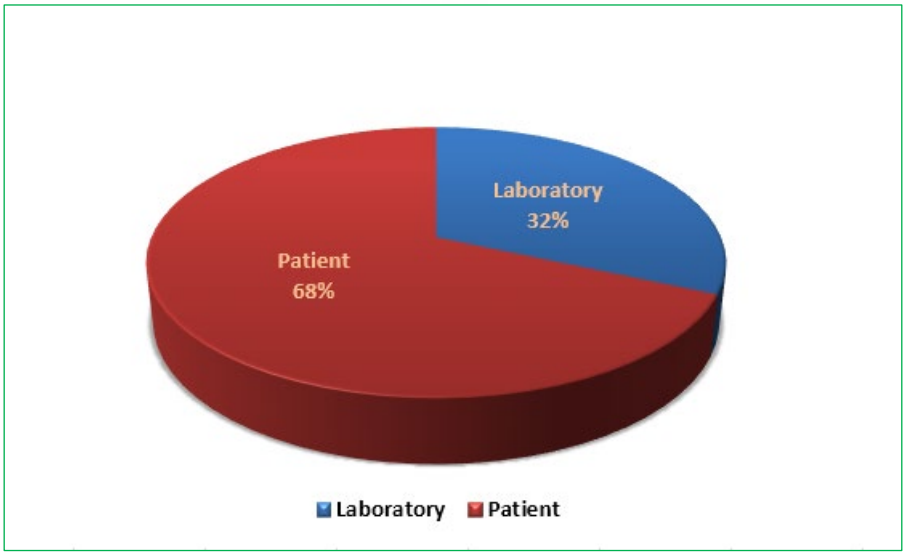


Figure 3.3: Patient specimens emanating from hospitals and laboratories

Table 3.4 and Figure 3.4 summarize the distribution of specimens collected from the various hospital departments and laboratories. The highest numbers were collected from Outpatients and Inpatients (hospitalized patients), contributing respectively 307 (70%) and 107 (24%) of the total sample count of 442 (100%). The lowest contribution was from ICUs and newborn groups, which respectively provided 4 (1%) and 24 (5%) samples of the total of 442 (100%) specimens.

Table 3.4: Distribution of patient samples collected from hospital departments and laboratories

Department	Number (n)	Percentage (%)
ICU	4	1%
Inpatients	107	24%
Newborns	24	5%
Outpatients	307	70%
Total (n)	**442**	**100%**

ICU=intensive care unit hospital patients=admitted patients with allocated beds; newborns=patients younger than 1 year; outpatients=day patients; not admitted into the hospitals.

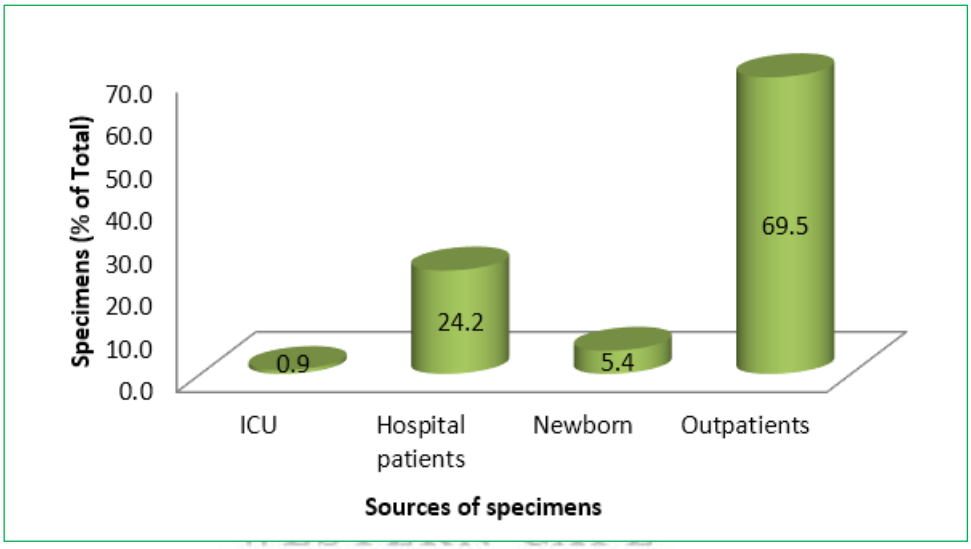
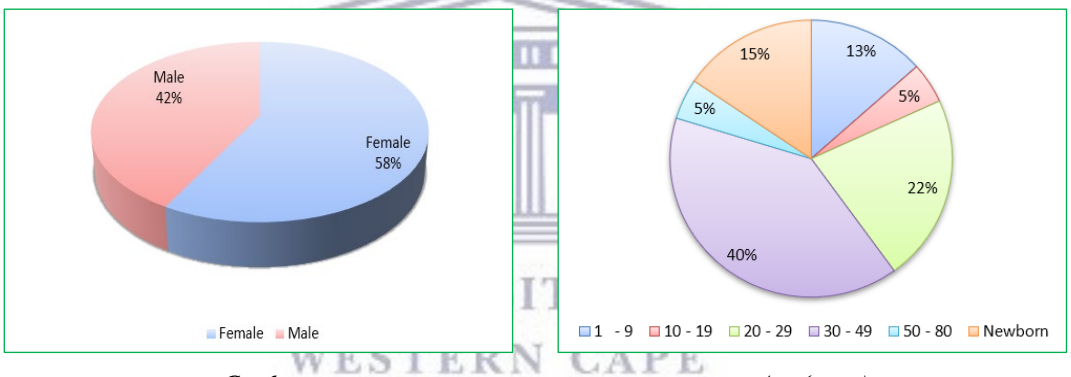


Figure 3.4: Comparison between the number of samples collected from hospital patients and laboratories

Table 3.5 and Figure 3.5 summarize the number of samples collected and tested according to gender and age. From a gender perspective, females contributed 256 (58%) of the total number of specimens (n=442), while males contributed 186 (42%). When considering the total number of specimens (n=442) in terms of age groups, the highest number of specimens were derived from the 30–49-year old group, namely 151 (39%), while the lowest contribution of 18 (5%) came from each of the age groups 10–19 and 50–80 years.

Table 3.5: Comparison of the number of samples collected and tested according to gender and age

Demographic variable		Number (n)	Percentage
Gender	**Female**	256	58%
	Male	186	42%
Age	**1-9 years**	51	13%
	10-19 years	18	5%
	20-29 years	83	22%
	30-49 years	151	39%
	50-80 years	18	5%
	Newborn (<1 year)	59	15%



Gender *Age (years)*

Figure 3.5: Comparison of the number of samples collected and tested according to gender and age

Table 3.6 and Figure 3.6 present data on the types of specimens collected and tested. The majority of specimens collected and tested were in the following order: urine (37.4%), swabs (31.5%), semen (17%) and cerebrospinal fluid (CSF) (9.2%), swabs from operations (2.4%). The remainder of samples, i.e., blood culture, eye swab, lung swab, sputum, stool, throat swab, and wound swab each constituted less than 1% of the total number of specimens collected and tested.

Table 3.6: Types of specimens collected and tested

Specimen	Frequency	Percent
Blood Culture	2	0.3%
Cerebrospinal fluid (CSF)	60	9.2%
Eye Swab	3	0.5%
Lung Swab	1	0.2%
Semen	113	17%
Sputum	2	0.3%
Stool	1	0.2%
Swab	206	31.5%
Swab from Operation	16	2.4%
Throat Swab	1	0.2%
Urine	245	37.4%
Wound Swab	5	0.8%
Total	**655**	**100%**

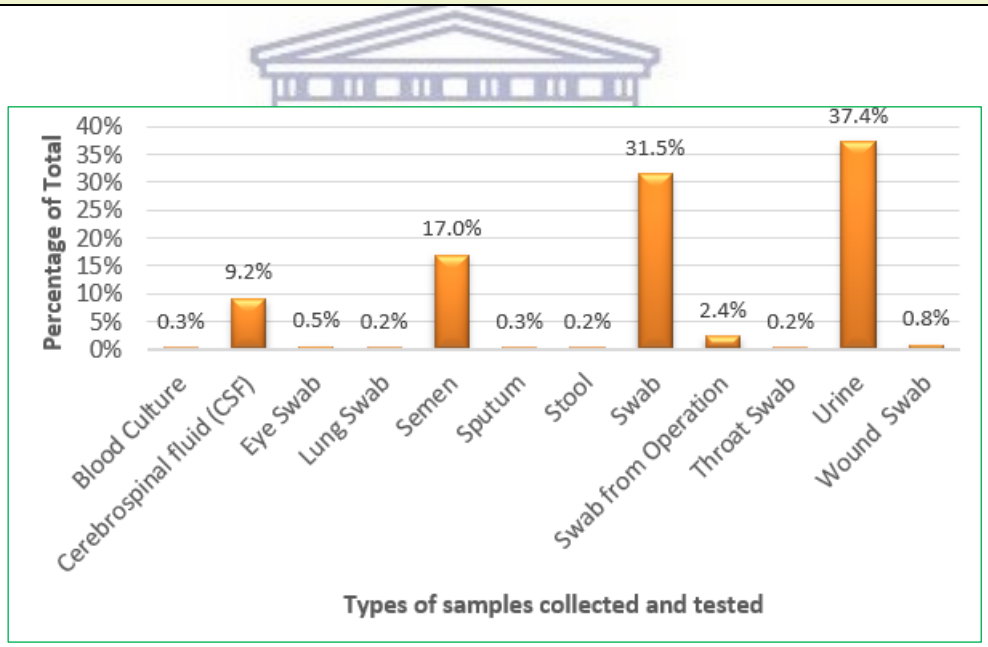


Figure 3.6: Types of specimen collected and tested

3.1.3 Bacterial and Fungal Growth Classification

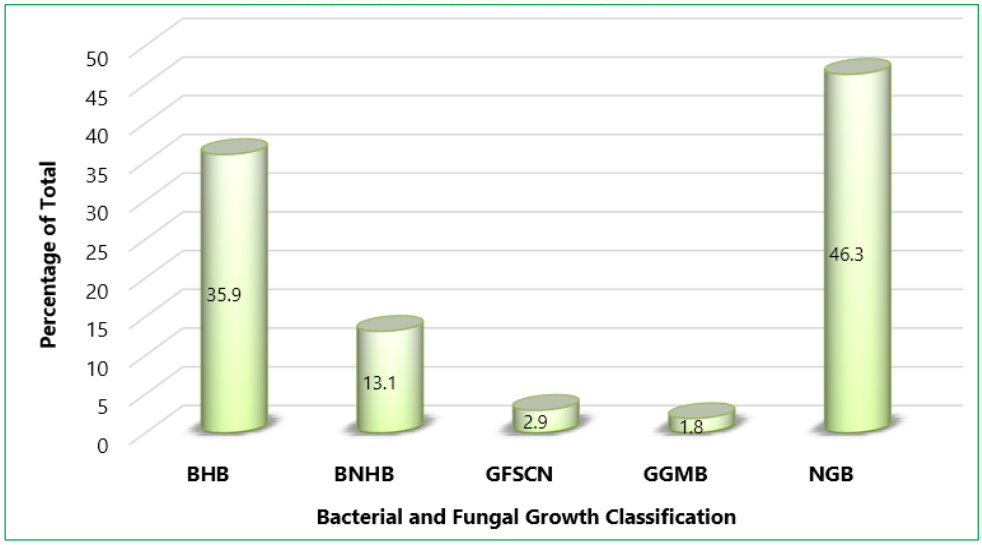
3.1.3.1 Blood Agar Culture: Bacterial and Fungal Growth Classification

Table 3.7 and Figure 3.7 summarize data on the growth differentials of bacterial and fungal species in the samples tested. Out of a total number of 657 specimens, the highest number of

specimens with bacteria present were found in NGB (No Growth Bacteria) and BHB (Bacteria No Haemolytic Blood), where it occurred respectively in 304 (46.3%) and 236 (35.9%) of the specimens. The lowest number of specimens with bacteria were noted in GFSCN (Growth Fungi, *Staphylococcus* Coagulase-Negative) and GGMB (Growth Gram-Mixed Bacteria), with respectively 19 (2.9%) and 12 (1.8%) of the total 657 samples.

Table 3.7: Blood agar culture: Bacterial and fungal growth classification

Blood Agar Culture	Frequency	Percent
BHB: Bacteria Haemolytic Blood	236	35.9%
BNHB: Bacteria No Haemolytic Blood	86	13.1%
GFSCN: Growth Fungi, *Staphylococcus* Coagulase-Negative	19	2.9%
GGMB: Growth Gram-Mixed Bacteria	12	1.8%
NGB: No Growth Bacteria	304	46.3%
Total	**657**	**100%**



BHB: Bacteria Haemolytic Blood; **BNHB:** Bacteria No Haemolytic Blood; **GFSCN:** Growth Fungi, *Staphylococcus* Coagulase-Negative; **GGMB:** Growth Gram-Mixed Bacteria; **NGB**: No Growth Bacteria

Figure 3.7: Blood agar culture: Bacterial and fungal growth classification

3.1.3.2 Comparative Number of Observations of Gram-stain, Catalase and Coagulase

Table 3.8 and Figure 3.8 show the relationship between G+ (Gram-positive) and G- (Gram-negative) for each of the Gram-stain, catalase and coagulase tests. The number of positive samples of the Gram-stain test was 195 (55%), while the number of negative samples of the Gram-stain test was 161 (45%). The positive samples of the catalase test numbered 301 (93%), while the number of catalase-negative samples was 21 (7%). Furthermore, the number of the positive samples of the coagulase test was 84 (49%), and the number of coagulase-negative samples was 87 (51%).

Table 3.8: Comparative number of observations of Gram-stain, catalase and coagulase

Test		G-	G+
Gram-stain	**n**	161	195
	%	45%	55%
Catalase	**n**	21	301
	%	7%	93%
Coagulase	**n**	87	84
	%	51%	49%
Gram-positive (G+) and Gram-negative (G-)			

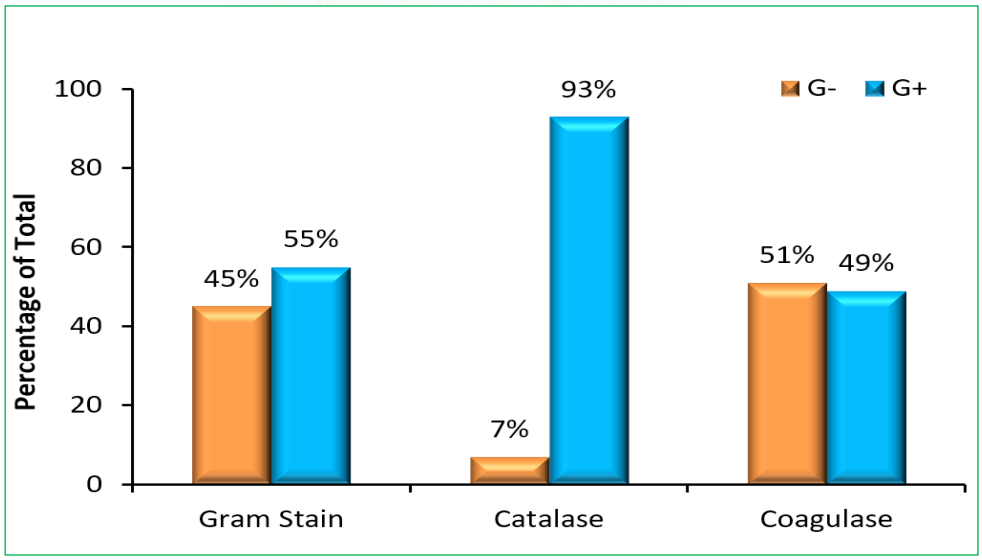


Figure 3.8: Comparative number of observations of Gram-stain, catalase and coagulase

3.1.3.3 Diagnostic Profile of the Bacterial and Fungal Species in Specimens

Table 3.9 and Figure 3.9 show the diagnostic profile of the bacteria and fungal species in specimens tested. The highest numbers are for NGB, BGN and *S. aureus* with respective counts of 289 (44.70%), 161 (25%) and 87 (13.40%), from a total of 647 specimens. On the other hand, the lowest numbers are for *Candida*, *Streptococcus* and GF (fungal growth) with respective counts of 1 (0.2%), 14, (2.20%) and 15 (2.30%) of a total of 647 specimens.

Table 3.9: Diagnostic profile of the different bacterial and fungal species in specimens tested

Diagnostic	Number (n)	Percentage
BGN	161	25.0%
Candida	1	0.2%
S. aureus	87	13.4%
GF	15	2.3%
NGB	289	44.7%
SCN	80	12.4%
Streptococcus	14	2.2%
Total	**647**	**100%**

BNB=growth negative bacteria; *S. aureus*=Growth *Staphylococcus* coagulase positive; NGB=sterile no growth bacteria; SCN=*Staphylococcus* coagulase negative, *Streptococcus* spp.

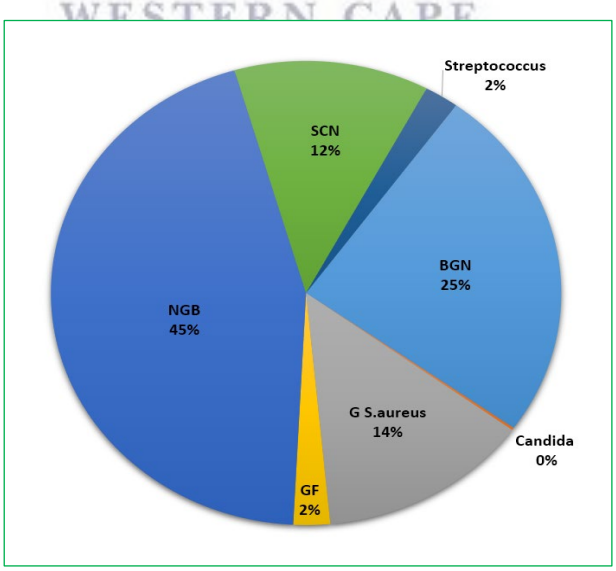


Figure 3.9: Diagnostic profile of the different bacterial and fungal species in specimens tested

3.1.3.4 Growth of Bacteria on Mueller-Hinton Agar (MHA) Plates

Table 3.10 shows that the growth of bacteria on Mueller-Hinton agar was found to be 100% *S. aureus*.

Table 3.10: Growth of bacteria on Mueller-Hinton agar plates

Diagnostic	Number	Percentage
S. aureus	73	100
Total	**73**	**100**

3.1.4 Antibiotic Sensitivity and Resistance Profiles for *Staphylococcus aureus*

3.1.4.1 *Staphylococcus aureus* Antibiotic Sensitivity and Resistance Profiles for Patient Samples

Table 3.11 shows the antibiotic resistance (R), sensitivity (S) and intermediate sensitivity (I) profiles of patient samples tested. Antibiotics analyzed were oxacillin, vancomycin, tetracycline, kanamycin, streptomycin erythromycin, gentamicin and amikacin. The highest sensitivities were towards vancomycin, amikacin, streptomycin and gentamicin, with respective counts of 70 (96%), 65 (89%), 60 (82%) and 59 (81%), while lower sensitivities were noted for erythromycin, oxacillin, kanamycin and tetracycline, with respective counts of 18 (25%), 36, (49%), 36, (49%) and 37 (51%). The highest intermediate sensitivity count was for gentamicin, i.e., 3 (4%), while the lowest intermediate sensitivity counts were 1 (1%) for both amikacin and erythromycin, while there were no intermediate sensitivity counts for oxacillin and vancomycin.

In this study, it was found that the sensitivity counts of coagulase-positive (*S. aureus*) to gentamicin was 59 (81%), which is consistent with a study by Moorhouse et al. (1996) in that the sensitivity to gentamicin was observed to be 89% from a total of 1018 isolates; of this total, 361 were regarded as hospital acquired, 506 were community acquired and the sources of the remaining 192 isolates were unknown. However, in this study, it was found that sensitivity to

erythromycin had a count of 18 (25%), which does not agree with the findings of the study by Moorhouse et al. (1996) which determined the sensitivity to erythromycin to be 80%. The sensitivity of the methicillin-resistant strains to the other antibiotics tested, was generally low compared to methicillin-resistant *S. aureus* (MRSA).

In this study, it was found that the highest sensitivity was to the antibiotic vancomycin, which amounted to 96% and this is supported by a study by Leibler et al. (2017), who demonstrated that samples tested for antibiotic resistance to erythromycin, vancomycin, tetracycline was 81.2%, 0% and 0%, respectively, and in agreement with the observation of 74% resistance to erythromycin in the present study.

Table 3.11: *Staphylococcus aureus* antibiotic sensitivity and resistance profiles for patient samples

Antibiotic		Intermediate sensitivity (I)	Resistance (R)	Sensitivity (S)
Oxacillin	**n**	0	37	36
	%	0%	51%	49%
Vancomycin	**n**	0	3	70
	%	0%	4%	96%
Tetracycline	**n**	0	36	37
	%	0%	49%	51%
Erythromycin	**n**	1	54	18
	%	1%	74%	25%
Gentamicin	**n**	3	11	59
	%	4%	15%	81%
Kanamycin	**n**	0	37	36
	%	0%	51%	49%
Streptomycin	**n**	0	13	60
	%	0%	18%	82%
Amikacin	**n**	1	7	65
	%	1%	10%	89%

Moreover, MRSA showed 37.5% resistance to vancomycin. Generally, antibiotics are regarded as expensive drugs in Uganda (Seni et al., 2013). In this study, the sensitivity to vancomycin

was 96% and the resistance was 4%, and sensitivity to erythromycin was 25% and that the resistance was 74% and intermediate sensitivity was 1%. The results of this study are consistent with those of a study by Ahmed et al. (2012) which was done in Tripoli, Libya. They found that the sensitivity to vancomycin was 88% and MRSA resistance to vancomycin was 12%, using the disc spread method of the antibiotic vancomycin. A further finding from this study was the high resistance of 74% to erythromycin; this finding is congruent with the study by Ahmed et al. (2012) in which the proportion of resistance to erythromycin was also reported to be 74%.

S. aureus MRSA was tested by PCR and the rates were 88.2% for erythromycin, 58.8% for gentamicin; these results signal that vancomycin appears to be the only antimicrobial factor to considered for antimicrobial therapeutic regimens that target MRSA (Baby et al., 2017; Ojulong et al., 2009; Smith et al., 2017). Also, vancomycin was validated for its efficacy to eliminate MRSA (Ahmed et al., 2012). The results of the present study corroborate those of a study conducted by Prakash et al. (2007) in which they obtained the highest sensitivity to the vancomycin with a count of 90.4% and a resistance to the antibiotic vancomycin which reached a count of 9.6%. However, not all the studies agree with the results of this current study, with several studies having proved that MRSA strains were 100% sensitive to vancomycin (Chambers, 1997; Rajaduraipandi et al., 2006; Sklyar et al., 2018).

Several studies in Libya have also proved MRSA sensitivity to the antibiotic vancomycin and this is therefore a good and effective treatment to eliminate these bacteria (Ahmed et al., 2010). The present results match those of Ahmed et al. (2012) who recorded MRSA strains with a percentage resistance to the antibiotic vancomycin, reaching 12%, and not in agreement with the results of Ahmed et al. (2010) who recorded strains of MRSA with a percentage resistance to vancomycin reaching 27%. In this current study, an increased count in the resistance to erythromycin was found to be 74%; this is consistent with the results of Ahmed et al. (2010) who found a count of 63.2% for hospitals in Tripoli.

Another study found that resistance to vancomycin and erythromycin totalled 17.7% and 38.7%, respectively (Buzaid et al., 2011). The plausible reason for this is the excessive and random use of antibiotics without prescription. In this study, vancomycin resistance was 4% using the disc spread of the antibiotic. Our results do not agree with the study by Salman et al. (2018) which found 100% MRSA sensitivity to vancomycin and amikacin. MRSA resistance to oxacillin was 100%, while MRSA was 100% sensitive to vancomycin and amikacin. In another study, 60 (50%) of MRSA strains were resistant to vancomycin and 70 (58.3%) to erythromycin (Hassanzadeh et al., 2013). Also, *S. aureus* were susceptible to kanamycin and amikacin (Edslev et al., 2018).

Yet another study by Prakash et al. (2007) determined an erythromycin count which was convergent with the highest MRSA resistant count observed for resistance towards erythromycin, namely 74%. The reason for the increased high rate of resistance to the antibiotic can be ascribed to the use of mainly β-lactam antibiotics to treat most infections caused by *S. aureus* which allows methicillin-resistant *S. aureus* (MRSA) to proliferate in the presence of β-lactam antibiotics, due to a gene that encodes the β-lactam-insensitive penicillin-binding protein PBP2a (Foster, 2018). Erythromycin resistance may be the result of the large number of doctors who overprescribed this antibiotic.

Our results agree with the 86.7% resistance to erythromycin reported by Orrett et al. (2006) and further correspond with the MRSA results reported by Wang et al. (2004) found in children in Taiwan where the resistance to erythromycin count was 92%. In addition, this result is also corroborated by the study of Ahmed et al. (2012), which determined the count of resistance to erythromycin as 74%. One of the findings of the current study is that MRSA had a resistance count to gentamicin of 15%, a sensitivity count of 81%, and an intermediate sensitivity of 4%, of a total of 73 strains of *S. aureus*. This result does not agree with an earlier report by Rajaduraipandi et al. (2006) where the gentamicin resistance rate was stated confirmed as 63.2%. Also, it is not consistent with the study by Sifaw et al. (2013) who found 68.54% *S.*

aureus resistance to methicillin, but no vancomycin resistance of *S. aureus*. In addition, other studies by Vindel et al. (Vindel et al., 2009) and Sklyar et al. (2018) found resistance to gentamicin (20%), erythromycin (66.7%) and methicillin (29.2%). As indicated above, the increased use of β-lactam antibiotics to treat the majority of infections caused by *S. aureus* allows methicillin-resistant *S. aureus* (MRSA) to replicate in the presence of β-lactam antibiotics, due to a gene that encodes the β-lactam-insensitive penicillin-binding protein PBP2a (Foster, 2018).

3.1.4.2 Detection of MRSA-Sensitive and-Resistant Profiles Using the EZY MIC Paper Strip

Table 3.12 shows the Minimum Inhibitory Concentration (MIC) test results of the EZY MIC Paper Strip for oxacillin. All 36 isolates were resistant to oxacillin. The rate was counted at 33.1 (91%). In addition, 27.8% of MRSA strains were resistant to oxacillin at concentrations (1 µg/ml; 0.8 µg/ml), 23 (63.88%) at concentrations (2 µg/ml; 0.4 µg/ml), 6 (16.6%) strains MRSA were resistant to oxacillin at concentrations (16 µg/ml; 32 µg/ml).

Table 3.13 shows 43 swabs of the various devices and incubators of the child care unit (ICU). We found 5 MRSA strains resistant to oxacillin with a rate of 11.62%. We found 3 MRSA strains expressing genes *mecA1*, *mecA2* by PCR and two MRSA in the children incubator in the ICU and one from the ground room. In the Surface Suction Devices, MRSA strains resistant to oxacillin were found, but these did not correlate with *gene mecA1* and *mecA2* by PCR. It accounted for 25% of the total MRSA from care units of the Central Hospital Misurata. Table 3.14 shows swabs of the various devices of care units. We found 2 MRSA strains resistant to oxacillin with ratio count of 4.65 in ICU. One MRSA of the ground was resistant to oxacillin. We found the gene *mecA1, mecA2* by PCR. It accounted for 50% of the total *S. aureus*. We found another MRSA on the door handle of a care unit. It was resistant to oxacillin and positive for *mecA1* and *mecA2* by PCR. It accounted for 50% of the total *S. aureus*. The two MRSA was sensitive to vancomycin, tetracycline, streptomycin and amikacin.

Table 3.12: Detection of MRSA-sensitive and-resistant profiles using the EZY MIC Paper Strip

Minimum Inhibitory Concentration (MIC) in µg/l at Oxacillin concentrations in µg/ml																
0.03	0.06	0.12	0.25	0.5	1	2	4	4	4	8	16	32	128	164	256	Ox*
18	18	18	18	18	17	13	8			6	3	3	3	3	3	3
1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	4	4	3	0	0	0	0	0	0	0	0	0	0
11	11	11	11	11	9	8	4	4	4	3	2	2	2	2	2	2
1	1	1	11	1	1	1	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
36	36	36	36	36	33	23	23	23	23	10	6	6	6	6	6	6
100%	100%	100%	100%	100%	91.66%	63.88%	63.88%	63.88%	63.88%	27.77%	16.66%	16.66%	16.66%	16.66%	16.66%	16.66%

*No zone would be obtained for MRSA on side coated with oxacillin; Technical Bulletin: http://himedialabs.com/TD/EM063.pdf; **Source:** HiMedia Laboratories Pvt. Ltd. A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com Website: www.himedialabs.com; **Reference**: Performance standards of Antimicrobial Disc Susceptibility Tests, M100- S21 CLSI Vol. 31 No.1, Jan 2011.

Table 3.13: Isolation of MRSA from care units of Central Hospital Misurata

Source	Total cases	Oxacillin		Vancomycin		Tetracycline		Erythromycin		Gentamicin		Kanamycin		Streptomycin		Amikacin		PCR (R)(+)	PCR (-)
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R		
Surface Suction Device	1 25%	0 0%	1 25%	1 25%	0 0%	1 25%	0 0%	0 %	1 25%	1 25%	0 0%	1 25%	0 0%	1 25%	0 0%	1 25%	0 0%	0 0%	1 25%
Ground Room (3)	1 25%	0 0%	1 25%	1 25%	0 0%	1 25%	0 0%	0%	1 25%	1 25%	0 0%	1 25%	0 0%	1 25%	0 0%	1 25%	0 0%	1 25%	0
Fawzia Attia ICU Incubator	1 25%	0 0%	1 25%	0	1 25%	1 25%	0 0%	1 25%	0 0%	1 25%	0%	1 25%	0 0%	1 25%	0 0%	1 25%	0 0%	1 25%	0 0%
(Antasar Ali ICU Incubator	1 25%	0 0%	1 25%	1 25%	0%	1 20%	0 0%	1 20%	0 0%	1 20%	0%	1 20%	0 0%	1 20%	0 0%	1 20%	0 0%	1 20%	0 0%
Total of All Cases	4 100%	0 0%	4 100%	3 75%	1 25%	4 100%	0 0%	2 50%	2 50%	4 100%	0	4 100%	0 0%	4 100%	0 0%	4 100%	0 0%	3 75%	1 25%

S=sensitive; R=resistant; PCR=Polymerase Chain Reaction

Table 3.14: Isolation of MRSA from hospital units of the National Cancer Institute

Total Cases		Oxacillin		Vancomycin		Tetracycline		Erythromycin		Gentamicin		Kanamycin		Streptomycin		Amikacin		PCR (R)(+)
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
Ground	1 50%	0	1 50%	1 50%	0	1 50%	0	0	1 50%	0	1 50%	1 50%	0	1 50%	0	1 50%	0	1 50%
Door Handle	1 50%	0	1 50%	1 50%	0	1 50%	0	0	1 50%	1 50%	0	0	1 50%	1 50%	0	1 50%	0	1 50%
Total of All Cases	2 100%	0	2 100%	2 100%	0 0%	2 100%	0	0	2 100%	1 50%	1 50%	1 50%	1 50%	2 100%	0	2 100%	0	2 100%

Table 3.15 summarizes swab samples collected from various devices used in ICU, laboratories and the surgical department. We found 11 MRSA strains sensitive to oxacillin, streptomycin, amikacin, vancomycin and tetracycline with the ratio count 11 (100%) ,11 (100%), 11 (100%), 10 (90.90%) and 7 (63.63%), respectively, of the total *S. aureus* collected. In this study, we aimed to establish how the transfer and contamination with *S. aureus* might have occurred. For this, we took 206 swabs from laboratory equipment and the intensive care unit (ICU) room of the Central Hospital and Oncology Unit.

We found 6 strains resistant to the antibiotic oxacillin and MIC—4 of them with 2 strains isolated from the children incubators of and one strain from the ground of the ICU of Central Hospital Misurata were resistant to the antibiotic oxacillin, positive to PCR gene *mecA1* and *mecA2*, but one strain was negative, non-identical to the PCR non-carrying gene *mecA1, mecA2*, and one MRSA isolated strain from the suction device.

Also, in the Hospital National Cancer Institute Misurata we isolated two MRSA strains, one from the door handle of the ICU and another from the laboratory floor—both were resistant to oxacillin and fully matched the PCR analysis gene profiles for *mecA1 and mecA2*, agreeing with an earlier report by Spengler et al. (1978). In addition, 25 swab samples of horizontal surfaces, air and the environment were 14 (70%), suggesting potential ecological pollution of the isolation rooms, possibly contributing to endemic MRSA (Sexton et al., 2006).

The overall MRSA infection in our study was found to be 11.6%: 4.56% from care units of the Central Hospital Misurata, Hospital National Cancer Institute Misurata, which is in accord with a study by Kumari et al. (2008) in MRSA from ICU accounted for only 10% of all cases. Our findings corroborate earlier assertions by Kumari et al. (2008) and, as such, we can thus benefit from this study finding with the prospect of efficacy of good infection control in our hospitals. This is supported by Kato et al. (2018) who reported MRSA spread in a six-bed Newborn Instensive Care Unit (NICU), affecting 5 babies within 13 days of the emergency.

Table 3.15: Isolation of MRSA from hospital units and laboratories of the National Cancer Institute and Central Hospital Misurata

Total Cases	Oxacillin		Vancomycin		Tetracycline		Erythromycin		Gentamicin		Kanamycin		Streptomycin		Amikacin	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Central Hospital Misurata ICU Laboratory	4 36.36%	0	3 27.27%	1 9.09%	1 9.09%	3 27.27%	1 9.09%	3 27.27%	3 27.27%	1 27.27%	2 18.18%	2 18.18%	4 36.36%	0	4 36.36%	0
National Cancer Institute Misurata Laboratory ,	6 54.54%	0	6 54.54%	0	5 45.45%	1 9.09%	2 18.18%	4 36.36%	3 27.27%	3 27.27%	3 27.27%	3 27.27%	6 54.54%	0	6 54.54%	0
Surgical Department	1 9.09%	0	1 9.09%	0	1 9.09%	0	0	1 9.09%	1 9.09%	0	1 9.09%	0	1 9.09%	0	1 9.09%	0
Total	11 100%	0	10 90.9%	1 9.09%	7 63.63%	4 36.36%	3 27.27%	8 72.72%	7 63.63%	3 27.27%	6 54.54%	5 45.45%	11 100%	0	11 100%	0

In addition, prevalence of methicillin resistance was highest among *S. aureus* isolated from respiratory specimens. Also, MRSA appeared to be more prevalent in ICUs and operation wards than in other departments through a 12-month period of 1154 hospital-acquired contagion which accounted for 7% of all cases (Saderi et al., 2009). In another study, MRSA strains were tested in patients in ICUs with high mortality rates were of the order of 29.1% (Hanberger et al., 2011). Isolated patients were not at increased risk of oversedation compared with non-isolated patients. There was an association between isolation for MRSA colonization and long ICU stay (Searcy et al., 2018). In addition to MRSA, *E. coli* was the main cause of surgical site infections (Hidayatullah et al., 2018).

A recent study presented evidence that could revolutionize strategies to prevent bacterial spread from ICUs to reduce mortality rates (Pouwels et al., 2018). Another report recommended improving environmental cleaning, evaluating effectiveness of environmental cleaning and disinfection practices and empowering staff to observe and enforce hand-hygiene compliance (Huang et al., 2019). Furthermore, the promotion of a surveillance culture for microbial infections is encouraging (Southwick et al., 2017). We could benefit from our study in that we found the contamination of the surroundings in the ICUs of the Central Hospital Misurata which exceeded that of the ICU in National Cancer Institute Hospital Misurata.

3.1.5 Comparison of *Staphylococcus aureus* in Samples According to Gender

3.1.5.1 Comparison of *Staphylococcus aureus* in Specimens According to Gender

Table 3.16 shows that the difference in *S. aureus* between the number of samples collected from male and female patients is significant (Chi-Square=0.000). The numbers of samples collected from female patients as a percentage of total (male and female) were, in descending order: ear swab and throat swab (100%, respectively), urine (81.2%), wound swab (80%), CSF (74.6%), swab from operation (56.3%) and blood culture (50%).

By contrast, the numbers of samples collected from male patients as a percentage of total (male and female) were, in descending order: lung swab, semen, sputum, stool (100% each), blood culture (50%), swab from operation (43.8%), CSF (25.4%), wound swab (20%) and urine (18.8%).

Table 3.16: Comparison of *Staphylococcus aureus* in various specimens according to gender

Sample		Gender		
		Female	Male	Total
Blood Culture	n	1	1	2
	%	50.0%	50.0%	100%
CSF	n	44	15	59
	%	74.6%	25.4%	100%
Ear Swab	n	3	0	3
	%	100%	0.0%	100%
Lung Swab	n	0	1	1
	%	0.0%	100%	100%
Semen	n	0	113	113
	%	0.0%	100%	100%
Sputum	n	0	2	2
	%	0.0%	100%	100%
Stool	n	0	1	1
	%	0.0%	100%	100%
Swab from Operation	n	9	7	16
	%	56.3%	43.8%	100%
Throat Swab	n	1	0	1
	%	100%	0.0%	100%
Urine	n	194	45	239
	%	81.2%	18.8%	100%
Wound Swab	n	4	1	5
	%	80.0%	20.0%	100%
Total	n	256	186	442
	%	57.9%	42.1%	100%

Chi-Square Tests	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	224.746[a]	10	0.000
Likelihood Ratio	273,782	10	0.000
No. of Valid Cases	442		

3.1.5.2 Comparison of Blood Agar Cultures of Samples According to Gender

Table 3.17 shows the growth of bacteria in blood agar cultures from female and male patient samples and classified depending on bacteria haemolytic blood (BHB) and bacteria non-haemolytic blood (BNHB). The highest rate of BHB was observed in male patient samples, with a count of 27.1% of total specimens (n=186) while the lowest rate of BHB was noted in the female patient samples, with a count of 16.7% of total specimens (n=256). The highest count of BNHB in females was 10.2%, and GNB and GFSCN was 2%, while the lowest rate of BNHB in males was 5%. GNB and GFSCN were absent in the male specimens. The highest rate of NGB in females had a count of 30.5% while lowest rate of NGB was 10.0% in males.

Table 3.17: Comparison of blood agar cultures of samples according to gender

			Blood Agar Culture (BAC)					
			BHB	**BNHB**	**GFSCN**	**GNB**	**NGB**	**Total**
Gender	**Female**	**n**	74	45	1	1	135	256
		%	16.7%	10.2%	2%	2%	30.5%	57.9%
	Male	**n**	120	22	0	0	44	186
		%	27.1%	5.0%	0.0%	0.0%	10.0%	42.1%
	Total	**n**	194	67	1	1	179	442
		%	43.9%	15.2%	2%	2%	40.5%	100%

BHB=Bacteria haemolytic blood; BNHB=Bacteria no haemolytic blood; GNB=Gram-negative bacteria; GFSCN=Growth fungi and bacteria (*Staphylococcus*) coagulase negative; NGB=No growth bacteria

Chi-Square Test	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	224.746[a]	10	0.000
Likelihood Ratio	273.782	10	0.000
No. of Valid Cases	442		

Statistical analysis shows that the blood agar cultures between male and female patients differed significantly (Chi-Square=0.000). We found that the BHB in males was greater than that of females, with the highest BHB ratio in males 27.1%, while the lowest ratio in females was 16.7%.

3.1.5.3 Comparison of Mannitol Salt Agar Cultures of Samples According to Gender

Table 3.18 shows the growth of fermenting bacteria in mannitol salt agar (MSA) cultures from female and male patient samples and classified depending on bacteria fermentation (BFMSA) and bacteria non-fermentation (BNFMSA). Statistical analysis shows that the MSA cultures between male and female patients differed significantly (Chi-Square=0.007). The highest rate of BFMSA was observed in male specimens, with a count of 34.4% of the total specimens (n=186), while the lowest rate of BFMSA observed in females was 20.7% of the total specimens (n=256).

Table 3.18: Comparison of mannitol salt agar cultures of samples according to gender

			Mannitol Salt Agar (MSA)				
			BFMSA	**BNFMSA**	**GNB**	**NGB**	**Total**
Gender	**Female**	**n**	53	26	1	176	256
		%	20.7%	10.2%	0.4%	68.8%	100%
	Male	**n**	64	21	0	101	186
		%	34.4%	11.3%	0.0%	54.3%	100%
	Total	**n**	117	47	1	277	442
		%	26.5%	10.6%	0.2%	62.7%	100%

BFMSA=Bacteria fermentation mannitol salt agar (MSA); BNFMSA=Bacteria non-fermentation mannitol salt agar; GFSCN=Growth fungi and bacteria coagulase negative; GNB=Gram-negative bacteria=*Enterobacteriaciae*, *Pseudomonas;* NGB=No growth bacteria

Chi-Square Test	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	12.090[a]	3	0.007
Likelihood Ratio	12.385	3	0.006
No. of Valid Cases	442		

The highest rate of BNFMSA in males was 11.3%, while the lowest rate of BNFMSA was in females, namely 10.2% of the total specimens (n=256). The highest rate of GNB (Gram-negative bacteria=*Enterobacteriaciae*, *Pseudomonas*) was in females with a count of 0.4%,

while GNB was absent in the males. The highest rate of NGB (no growth bacteria) in females was 68.8%, while in males it was 54.3%. Our results detected bacteria by MSA in both female and male specimens. We found that the BFMSA in males (34.4%; 64 out of 186) is greater than that of females (20.7%; 53 out of 256). The highest count in males was presumably due to samples containing *S. aureus* in collected semen specimens (n=133), which were absent from female specimens (see Table 3.16). Also, the highest rate NGB in males and female was 54.3% and 68.8%, respectively, which may be ascribed to high NGB in CSF and urine, particularly in the female specimens (Table 3.16).

From a public and personal health perspective, there should be no growth of any bacteria in specimens of CSF, urine and blood. Sterile testing of the isolates with MSA and the coagulase test, including tube coagulase, showed reliable results for identification of *Staphylococcus aureus* in our study was found to be 26.5% overall of the BFMSA (Table 3.18), which is in accordance with the report by Kateete et al. (2010).

3.1.5.4 Coagulase Test Results of Samples According to Gender

Table 3.19 summarizes the *Staphylococcus* coagulase-positive and-negative results of specimens from females and males. The highest count (61.4%) of *Staphylococcus* coagulase-positive was observed in male samples while the highest count (43.8%) of *Staphylococcus* coagulase-negative was in females. The highest rate for *Staphylococcus* coagulase-negative was in females, with a count of 43.8%. The lowest rate of *Staphylococcus* coagulase-negative was 38.6% in males, due to *S. aureus* being more prevalent in males than females (see Table 3.16). The coagulase-positive *Staphylococcus* with count (57%) were MRSA and (43%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). Thus, the early detection and diagnosis of MRSA is critical for prevention and control of acquired infection (Alzohairy, 2011). Accordingly, we can benefit in the clinical setting from testing and diagnosing *Staphylococcus aureus* using the coagulase test.

Table 3.19: Coagulase test results of samples according to gender

			Coagulase Test		
			Negative	**Positive**	**Total**
Gender	**Female**	**n**	28	36	64
		%	43.8%	56.3%	100%
	Male	**n**	22	35	57
		%	38.6%	61.4%	100%
	Total	**n**	50	71	121
		%	41.3%	58.7%	100%

Coagulase Negative=*Staphylococcus Coagulase negative*

Pearson Chi-Square	**Value**	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**
	0.330[a]	1	0.566		
Continuity Correction[b]	0.152	1	0.697		
Likelihood Ratio	0.331	1	0.565		
Fisher's Exact Test				0.584	0.349
No. of Valid Cases	121				

The statistical analysis shows the difference between male and female patient samples with regard to the coagulase test is insignificant for Fisher's Exact Test (p=0.349) and the Pearson Chi-Square Test (p=0.566). Thus, no variation was observed in the spread of MRSA among *S. aureus* from females or males using the coagulase test. This finding is congruent with a study by Buzaid et al. (2011).

3.1.5.5 Bacterial Diagnostic Test Results of Samples According to Gender

Table 3.20 compares the detection of bacteria in and female and male specimens (n=435). The highest rate of BGN, NGB and *S. aureus* was observed in males with counts of 39.2%, 23.8% and 19.3%, respectively. In males (n=181), the rates of SCN 12.2% (n=22) and *Streptococcus* 5.5% (n=10) were observed, but *Candida* was absent. The highest rates of BGN, NGB and *S. aureus* were detected in male specimens with respective counts of 39.2%, 23.8% and 19.3%. The highest rates of BGN and NGB were observed in females with counts of 23.6% and 49.2%,

respectively. However, the rates of *Candida*, *S. aureus*, SCN and *Streptococcus* were 0.4%, 14.2%, 11% and 1.6%, respectively of the total number of female samples (n=254). The elevated rates of NGB (49.2% in females) may due to the high prevalence of NGB observed in CSF (74.6%) and urine (81.2%) specimens as indicated Table 3.16. Ideally, no growth of any bacteria should be detected in sterile CSF, urine and blood specimens. In Table 3.20, the rate of *S. aureus* in the males (19.3%) exceeding that of females (14.2%) may be expected since *S. aureus* was positively identified in all (n=113, 100%) semen specimens collected and analyzed (see Table 3.16).

Table 3.20: Bacterial diagnostics of laboratory and hospitals samples according to gender

			Diagnostic						
			BGN	***Candida***	**G *S. aureus***	**NGB**	**SCN**	***Streptococcus***	**Total**
Gender	**Female**	**n**	60	1	36	125	28	4	254
		%	23.6%	0.4%	14.2%	49.2%	11.0%	1.6%	100%
	Male	**n**	71	0	35	43	22	10	181
		%	39.2%	0.0%	19.3%	23.8%	12.2%	5.5%	100%
	Total	**n**	131	1	71	168	50	14	435
		%	30.1%	0.2%	16.3%	38.6%	11.5%	3.2%	100%

BGN=*Enterobacteriaceae+Pseudomonas; S. aureus=Staphylococcus* coagulase-positive; NGB=sterile no growth bacteria; SCN=*Staphylococcus* coagulase-negative

Chi-Square Test	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	34.625[a]	5	0.000
Likelihood Ratio	35.888	5	0.000
Fisher's Exact Test	35.177		0.000
No. of Valid Cases	435		

[a]2 cells (16.7%) have expected count less than 5. The minimum expected count is 0.42.

Bacterial infections in both female and male patients are mainly due to *S. aureus.* We found high *S. aureus* infection in the males (19.3%, i.e., in 35 of the 181 specimens tested), while

females had lower infection rate (14.2%; 36 out of a total of 435 specimens). This observation was not in agreement with a study conducted by Buzaid et al. (2011) in which it was found that 31% of specimens tested positive for MRSA. No variation was observed in the spread of MRSA among *S. aureus* from females or males or from different age groups. In our study, the BGN rate was (31.1%) of the total number (n=435). Our results corroborate the findings of Shakya et al. (2017), in that 365 (80.9%) of the specimens showed *E. coli* and *Klebsiella pneumoniae* infections. In addition, Balan (2013) found 174 *Enterobacteriaceae and S. aureus.*

Moreover, we found *S. aureus* and SCN rates of 16.3% and 11.5%, respectively, in all samples tested (n=435; Table 3.20), which lends credence to the MRSA rate of 68.4% reported by Tadesse et al. (2018). By contrast, a study by Sexton et al. (2006) found that the total nosocomial bacteraemia offensive rate was 4.1 cases per 1,000 hospital patients. In addition, in 25 samples of patients and samples of surfaces, air and the environment, 14 (70%) samples from patient showed bacterial infections.

We also found *Streptococcus* (3.2%) out of the total number of samples (n=435). This corresponds with a study by Ohkoshi et al. (2018) which found *Streptococcus pneumonia*e strains from sputum and blood collected from a patient with disseminated intravascular coagulation in Sapporo City, Japan. Furthermore, Ghosh et al. (2017) reported a 7.7% positive diagnosis for *Candida* in 104 specimens collected from women with cervical cancer. Moreover, analyzes for *Staphylococcus aureus*, MRSA and *Candida albicans* showed 22 positives for *Candida albicans* out of 29 samples collected from adult patients whose dentures carried *Staphylococcus* and *Candida* species.

3.1.5.6 *S. aureus* Oxacillin-Resistant and-Sensitive Profiles According to Gender

Table 3.21 shows the oxacillin-resistant *S. aureus* (OXRSA) and oxacillin-sensitive *S. aureus* (OXSSA) profiles for females and males. The highest rate of OXRSA was observed in males

(61.5%; n=16) out of a total of 26 samples (i.e., n=26) whereas the observed OXRSA for females was 44.1% (i.e., 15 out of 34 samples). The highest rate for OXSSA was 55.9% for females (i.e., 19 out of 34 samples), while the OXSSA rate in males showed a lower rate (38.5%), i.e., 10 out of 26 samples).

Statistical analysis of the difference between male and female patients with respect OXRSA and OXSSA proved insignificant (Chi-Square=0.181). Nevertheless, our study shows a high incidence of OXRSA in both males and females (61.5% and 44.1%, respectively). This finding agrees with a study conducted by Jindamwar et al. (2016) which found MRSA in males and female at rates of 57% and 52%, respectively.

Table 3.21: Comparison of *S. aureus* resistance and sensitivity to oxacillin according to gender

			Oxacillin		
			OXRSA	**OXSSA**	**Total**
Gender	**Female**	**n**	15	19	34
		%	44.1%	55.9%	100%
	Male	**n**	16	10	26
		%	61.5%	38.5%	100%
	Total	**n**	31	29	60
		%	51.7%	48.3%	100%

OXRSA=Oxacillin-Resistant *S. aureus*; OXSSA=Oxacillin-Sensitive *S. aureus*

Pearson Chi-Square	**Value**	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**
	1.791[a]	1	0.181		
Continuity Correction[b]	1.161	1	0.281		
Likelihood Ratio	1.802	1	0.179		
Fisher's Exact Test				0.203	0.141
No. of Valid Cases	60				

Moreover, our study shows a high incidence of OXRSA infection in both male and female patients in the hospitals studied, and OXRSA may be transmitted between patients. This

observation further agrees with a study that MRSA may be found in the hospital environment (French et al., 2004). In addition, it bolsters a previously observed median range of 10-65% for MRSA within isolates of *S. aureus* (Buzaid et al., 2011). Similarly, our results agree with the study by Tadesse et al. (2018) who found an OXRSA rate of 68.4%.

It is expected that the increased use of β-lactam antibiotics to treat infections caused by *S. aureus* allows methicillin-resistant *S. aureus* (MRSA) to proliferate in the continued presence of β-lactam antibiotics. MRSA correlates with upregulation of the gene that encodes the β-lactam-insensitive penicillin binding protein PBP2a (Foster, 2018). Our study of OXRSA was also consistent with a study by Arora et al. (2010) which found such strains at a rate of 46% in patients. The spread of MRSA was found to be 19% of a total of 569 specimens collected and tested from among health care workers in Tripoli, Libya. The high rate of MRSA in our study was presumably also due to a compromise in the state of aseptic, sterilization and disinfection, isolation of MRSA patients and hand washing procedures (Romaniszyn et al., 2014).

3.1.5.7 *S. aureus* Vancomycin-Resistant and-Sensitive Profiles According to Gender

Table 3.22 shows the vancomycin-resistant *S. aureus* (VRSA) and vancomycin-sensitive *S. aureus* (VSSA) profiles for females and males. Males had the highest VRSA rate (3.8%, i.e., 1 out of 26 samples tested), whereas VRSA was absent in female samples (0.0%, 0 out of 34 samples). However, the highest VSSA rate was observed in female samples (100%, i.e., 34 out of 34 samples tested positive), while the VSSA rate in males was equally high at 96% (59 out of 60 samples were sensitive to the antibiotic).

The statistics show that the difference between male and female patients with respect to VRSA and VSSA is insignificant (Chi-Square=0.249). Our results indicate high rates of VSSA in both male and female specimens (i.e., 96.2% and 100%, respectively), and agrees with Tiwari et al. (2008) who reported that among 783 isolates of *S. aureus*, 301 (38.44%) were methicillin-

resistant, of which 217 (72.1%) were found to be multidrug-resistant. Almost all MRSA strains were resistant to penicillin, 95.68% were resistant to cotrimoxazole, 92.36% were resistant to chloramphenicol, 90.7% were resistant to norfl oxacin, 76.1% were resistant to tetracycline, and 75.75% were resistant to ciprofl oxacin. Vancomycin was the most effective drug, with only 0.33% of MRSA strains being resistant to it. By contrast, Orrett & Land (2006) found 78.7% VRSA. Our study results are similar to a reported VRSA of 1.7% (Roberts et al., 2018) and a tetracycline-resistant *S. aureus* (TRSA) in none of 13 samples tested (Hassanzadeh et al., 2013).

Table 3.22: Comparison of *S. aureus* resistance and sensitivity to vancomycin according to gender

			Vancomycin		
			VRSA	**VSSA**	**Total**
Gender	**Female**	**n**	0	34	34
		%	0.0%	100%	100%
	Male	**n**	1	25	26
		%	3.8%	96.2%	100%
	Total	**n**	1	59	60
		%	1.7%	98.3%	100%

VRSA=Vancomycin-Resistant *S. aureus*; VSSA=Vancomycin-Sensitive *S. aureus*

Pearson Chi-Square	**Value**	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**
	1.330[a]	1	0.249		
Continuity Correction[b]	0.018	1	0.892		
Likelihood Ratio	1.695	1	0.193		
Fisher's Exact Test				0.433	0.433
No. of Valid Cases	60				

3.1.5.8 *S. aureus* Erythromycin-Resistant and-Sensitive Profiles According to Gender

Table 3.23 depicts erythromycin-resistant *S. aureus* (ERSA) and erythromycin-sensitive *S. aureus* (ESSA) profiles for females and males. The detectable levels of erythromycin

intermediate-sensitive *S. aureus* (EISSA) in both female and male specimens were very low or negligible, i.e., 2.9% (1 out of 34 samples) for females and 0% (0 out of 26 samples for males). In the case of ESSA, 20.6% (7 out of 34) female samples tested positive, whereas for males it was 23.1% (i.e., 6 out of 26 samples). Noteworthy, however, was the high rate of ERSA in both female (76.5%; 26 out of 34) and male (76.9%; 20 out of 26) specimens.

Table 3.23: Comparison of *S. aureus* resistance and sensitivity to erythromycin according to gender

			Erythromycin			
			EISSA	**ERSA**	**ESSA**	**Total**
Gender	**Female**	**n**	1	26	7	34
		%	2.9%	76.5%	20.6%	100%
	Male	**n**	0	20	6	26
		%	0.0%	76.9%	23.1%	100%
	Total	**n**	1	46	13	60
		%	1.7%	76.7%	21.6%	100%

EISSA=erythromycin-intermediate-sensitive *S. aureus*; ERSA=erythromycin-resistant *S. aureus*; ESSA=erythromycin-sensitive *S. aureus*; *Staphylococcus aureus=S. aureus*

Chi-Square Test	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	0.807	2	0.686
Likelihood Ratio	1.178	2	0.555
No. of Valid Cases	60		

Statistical analysis did not show significant differences between male and female patients with respect to EISSA, ESSA and ERSA (Chi-Square=0.668). We found that the rates of ERSA in females and males were similar. The high ERSA may be due to several factors, including long-term antibiotic use without prescription, indiscriminate use of antibiotics, lack of realization and unethical treatment practices before coming to the hospital (Anupurba et al., 2003).

Moreover, patients are subjected to various accidents requiring hospitalization (Buzaid et al., 2011). Our results found ERSA to be 76.7% (i.e., 46 out of 60) of the total sample size for all

locations included in the testing. This corresponds with a study by Tiwari et al. (2008) in which an 85% ERSA (n=150) rate was documented. Our study agrees with that of Orrett & Land (2006) who reported an 86.7% ERSA. In addition, in our study, the highest ERSA (79.2% in males) is supported by another similar study (Leibler et al., 2017). Moreover, our results show a high rate of ERSA was in male and females with a count of (76.7%). By contrast, Jindamwar et al. (2016) observed a 41% ERSA rate in males and females which corroborates the work by Jamali et al. (2014), in which an ERSA of 39.5% (n=43) was established.

3.1.5.9 *S. aureus* Gentamicin-Resistant and-Sensitive Profiles According to Gender

Table 3.24 summarizes the gentamicin-resistant *S. aureus* (GRSA) and gentamicin-sensitive *S. aureus* (GSSA) profiles for females and males. Detection of gentamicin-intermediate-sensitive *S. aureus* (GISSA) was very low in both females (5.9%; 2 out of 34) and males (3.8%; 1 out of 26) and thus very low overall (5%; 3 out of a total of 60 samples tested). Similarly, the detection of gentamicin-resistant *S. aureus* (GRSA) in both female (14.7%; 5 out of 34) and male (7.7%; 2 out of 26) specimens was relatively low, but the overall rate of GRSA was arguably high (11.7%; 7 out of 60).

The highest rate for gentamicin-sensitive *S. aureus* (GSSA) in female specimens was 79.4% (27 out of 34) and in males it was 88.5% (23 out of 26), and overall it was 83.3% (50 out of 60). Statistical analysis of the difference between male and female patients with respect to GRSA, GISSA and GSSA showed no significance (Chi-Square=0.641). Our results indicate a much lower overall GRSA rate (11.7%; 7 out of 60 of specimens analyzed) than the 88% (n=150) reported by Tiwari et al. (2008). According to these authors, these results signal that vancomycin appears to be the only antimicrobial factor to inclusive therapy (Baby et al., 2017; Ojulong et al., 2009; Smith et al., 2017). Our results do not agree with the affirmation by Rajaduraipandi et al. (2006) that the GRSA rate was 63.2%. Our study shows a high overall GSSA rate in males and females (i.e., 83.3%) which is not consistent with the study of

Jindamwar et al. (2016) in which an overall GSSA rate in males and females was reportedly 40%.

Table 3.24: Comparison of *S. aureus* resistance and sensitivity to gentamicin according to gender

			Gentamicin			
			GISSA	**GRSA**	**GSSA**	**Total**
Gender	**Female**	**n**	2	5	27	34
		%	5.9%	14.7%	79.4%	100%
	Male	**n**	1	2	23	26
		%	3.8%	7.7%	88.5%	100%
	Total	**n**	3	7	50	60
		%	5.0%	11.7%	83.3%	100%

GISSA=gentamicin-intermediate-sensitive *S. aureus*; GRSA=gentamicin-resistant *S. aureus*; GSSA=gentamicin-sensitive *S. aureus*; *Staphylococcus aureus=S. aureus*

Chi-Square Test	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	0.888	2	0.641
Likelihood Ratio	0.919	2	0.632
No. of Valid Cases	60		

3.1.5.10 *S. aureus* Kanamycin-Resistant and-Sensitive Profiles According to Gender

Table 3.25 shows the kanamycin-resistant *S. aureus* (KRSA) and kanamycin-sensitive *S. aureus* (KSSA) profiles for females and males. The highest KRSA rate was observed for females (58.8%; 20 out of 34 samples) and for males it was 38.5% (10 out of 26 samples). Overall, both the KRSA and KSSA rates were 50.0% (i.e., 30 out of 60 samples). The statistics show that the difference between male and female patients with respect to KRSA and KSSA is insignificant (Chi-Square=0.406). We found that the KRSA in females (58.5%) was greater than that in males (38.5%), but generally these values do not agree with a recent similar study (Edslev et al., 2018), namely that out of a total of 126 samples, 98 were *mecA* positive and 28 were *mecA* negative *S. aureus* strains.

Table 3.25: Comparison of *S. aureus* resistance and sensitivity to kanamycin according to gender

			Kanamycin		
			KRSA	**KSSA**	**Total**
Gender	**Female**	**n**	20	14	34
		%	58.8%	41.2%	100%
	Male	**n**	10	16	26
		%	38.5%	61.5%	100%
	Total	**n**	30	30	60
		%	50.0%	50.0%	100%

KRSA=kanamycin-resistant *S. aureus*; KSSA=kanamycin-sensitive *S. aureus*; *Staphylococcus aureus*=*S. aureus*

Pearson Chi-Square	**Value**	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**
	0.690	1	0.406		
Continuity Correction[b]	0.241	1	0.621		
Likelihood Ratio	0.684	1	0.408		
Fisher's Exact Test				0.507	0.309
No. of Valid Cases	60				

3.1.5.11 *S. aureus* Streptomycin-Resistant and-Sensitive Profiles According to Gender

Table 3.26 displays the streptomycin-resistant *S. aureus* (SRSA) and streptomycin-sensitive *S. aureus* (SSSA) profiles for females and males. Males had the highest SRSA rate (23.1%; 6 out of 26 samples), while the value for females was slightly lower (14.7%; 5 out of 34). The SSSA rates for both females and males were very high, i.e., 85.3% (29 out of 34) and 76.9% (20 out of 26), respectively.

Likewise, the overall SSSA rate was vey high, i.e., 81.7% (49 out of 60). The statistics show that the difference between male and female patients with respect to SRSA and SSSA is insignificant (Chi-Square=0.406).

Table 3.26: Comparison of *S. aureus* resistance and sensitivity streptomycin according to gender

			Streptomycin		
			SRSA	**SSSA**	**Total**
Gender	**Female**	**n**	5	29	34
		%	14.7%	85.3%	100%
	Male	**n**	6	20	26
		%	23.1%	76.9%	100%
	Total	**n**	11	49	60
		%	18.3%	81.7%	100%

SRSA=streptomycin-resistant *S. aureus*; SSSA=streptomycin-sensitive *S. aureus*; *Staphylococcus aureus*=*S. aureus*

Pearson Chi-Square	**Value**	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**
	0.690	1	0.406		
Continuity Correction[b]	0.244	1	0.621		
Likelihood Ratio	0.684	1	0.408		
Fisher's Exact Test				0.507	0.309
No. of Valid Cases	60				

3.1.5.12 *S. aureus* Amikacin-Resistant and-Sensitive Profiles According to Gender

Table 3.27 summarizes the amikacin-resistant *S. aureus* (ARSA) and amikacin-sensitive *S. aureus* (ASSA) profiles for females and males. In female specimens, no amikacin-intermediate-sensitive *S. aureus* (AISSA) was detected, whereas in males the AISSA rate was 3.8% (1 out of 26 samples) compared to an overall AISSA rate of 1.6% (1 out of 60). In both females and males, the ASSA rates were substantially high, i.e., 85.3% (29 out of 34) and 88.5% (23 out of 26), respectively. The overall ASSA rate was also very high, i.e., 86.7% (52 out of 60). Despite the encouraging high ASSA rates, the ARSA rates for females and males, i.e., 14.7% (5 out of 34) and 7.7% (2 out of 26), respectively, as well as an overall rate of 11.7% (7 out of 60) may be a health concern. The statistics show no significant differences

between male and female patients with respect to ASSA and ARSA (Chi-Square=0.378).

Table 3.27: Comparison of *S. aureus* resistance and sensitivity to amikacin according to gender

			Amikacin			
			AISSA	**ARSA**	**ASSA**	**Total**
Gender	**Female**	**n**	0	5	29	34
		%	0.0%	14.7%	85.3%	100%
	Male	**n**	1	2	23	26
		%	3.8%	7.7%	88.5%	100%
	Total	**n**	1	7	52	60
		%	1.6%	11.7%	86.7%	100%

AISSA=amikacin-intermediate-sensitive *S. aureus*; ARSA=amikacin-resistant *S. aureus*; ASSA=amikacin-sensitive *S. aureus*; *Staphylococcus aureus=S. aureus*

Chi-Square Test	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	1.946	2	0.378
Likelihood Ratio	2.339	2	0.311
No. of Valid Cases	60		

We found that the overall AKRSA rate in females and males combined (11.7%) was not consistent with a value of 73% previously reported by Arora et al. (2010). Recently, evidence was presented for first time with respect to the anti-MRSA synergism of prenylflavonoids 1-4 with other antibacterial agents and this further reflects on MRSA to aminoglycosides (Zuo et al., 2018).

3.1.5.13 *S. aureus* PCR-Resistant and-Sensitive Profiles According to Gender

Table 3.28 summarizes the PCR analysis of methicillin (oxacillin) resistant *S. aureus* (MRSA) female and male specimens. The rates of PCR positive (PCR+) and PCR negative (PCR-) expression of the *mecA1* and *mecA2* genes were verified in the different specimens. The highest PCR+ rate was observed in male specimens, i.e., 100% of the total PCR+ number (n=15).

In female specimens, the rate was 93.4% (14 out of 15). The highest rate PCR- was (6.7%) from the females. The statistics showed no significant difference between male and female patients with respect to MRSA PCR+ and PCR- (Chi-Square=0.309).

Table 3.28: PCR analysis of methicillin (oxacillin) resistant *S. aureus* (MRSA) according to gender

			PCR		
			PCR-	**PCR+**	**Total**
Gender	**Female**	**n**	1	14	15
		%	6.7%	93.3%	100%
	Male	**n**	0	15	15
		%	0.0%	100%	100%
	Total	**n**	1	29	30
		%	3.3%	96.7%	100%

PCR-No genes *mecA1*, *mecA2* detected; PCR+=genes *mecA1*, *mecA2* detected; MRSA=Resistant Methicillin (Oxacillin) *S. aureus*

Pearson Chi-Square	**Value**	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**
	1.034	1	0.309		
Continuity Correction[b]	0.000	1	1.000		
Likelihood Ratio	1.421	1	0.233		
Fisher's Exact Test				1.000	0.500
No. of Valid Cases	30				

The results of the present study showed that the prevalence of traditional methods of disk diffusion and MIC strips, using oxacillin was efficient in the detection of MRSA because the error of PCR-, which accounted for 3.3% agreed with the study by Mohanasoundaram et al. (2008), which found one strain of the MSRSA isolate to show negative with PCR due to borderline resistance mediated by hyperproduction of beta-lactamases (Foster, 2018). PCR technology is an advantageous screening method for MRSA, but it is very expensive. Our results are consistent with Chambers's (1997), whereby the oxacillin PCR method was

validated to be the more reliable when compared with the disc diffusion sensitivity method. In addition to *SCCmec* variants, PCR-positive culture-negative results as well as patient samples contain densities of MRSA, which can be identified by PCR (Wolk et al., 2009). For resistant *S. aureus*, only 55% of all MRSA carrying the *mecA* gene tested positive with the disk diffusion method (Kareem, 2013). Antimicrobial susceptibility testing was conducted by agar disk diffusion where MRSA isolates carried five different *SCCmec* elements; this was the first report of *mecC*-MRSA isolated from animals in Austria (Schauer et al., 2018) that were tested for MRSA by four phenotypic methods. Clinical MRSA infection can be detected using PCR which has 88% sensitivity and 90% specificity (Chandak et al., 2018). Recently, PCR testing of pneumonia patients with MRSA has become increasingly popular (Baby et al., 2017).

3.1.5.14 Classification of Laboratory and Hospital Samples According to Gender

Table 3.29 shows a comparison of bacterial infections in female and male patient samples from three hospitals and a private laboratory. Samples collected from females had a higher infection rate (57.9%; 256 out of a total of 442 samples) than males (42.1%;186 out of a total number of 442).

Table 3.29: Patient samples collected from a laboratory and hospitals according to gender

Type of Sample		Gender		
		Female	Male	Total
Patient	n	256	186	442
	%	57.9%	42.1%	100%
Total	n	256	186	442
	%	57.9%	42.1%	100%

This finding does not agree with a study by Buzaid et al. (2011), in which 31% of samples were MRSA. No variation was observed in the spread of MRSA among *S. aureus* from females or males or from different age groups. This does not agree with a study by Ullah et al. (2018), which observed the highest prevalence in female infants (58.88%) vs male infants (41.11%).

3.1.5.15 Classification of Samples According to Specimen Type Collected

Table 3.30 shows the classification of the specimen type obtained from patients at the three hospitals and a private laboratory.

Table 3.30: Classification of laboratory and hospital samples according to specimen type collected

Type of Specimen		Type of Sample		
		Laboratory	Patient	Total
Blood Culture	n	0	2	2
	%	0.00%	100%	100%
CSF	n	0	60	60
	%	0.00%	100%	100%
Ear Swab	n	0	3	3
	%	0.00%	100%	100%
Lung Swab	n	0	1	1
	%	0.00%	100%	100%
Semen	n	0	113	113
	%	0.00%	100%	100%
Sputum	n	0	2	2
	%	0.00%	100%	100%
Stool	n	0	1	1
	%	0.00%	100%	100%
Swab	n	206	0	206
	%	100.00%	0.00%	100%
Swab from Operation	n	0	16	16
	%	0.00%	100%	100%
Throat Swab	n	0	1	1
	%	0.00%	100%	100%
Urine	n	0	245	245
	%	0.00%	100%	100%
Wound swab	n	0	5	5
	%	0.00%	100%	100%
Total	n	206	449	655
	%	100%	100.00%	100.00%

Chi-Square Test	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	655.000[a]	11	0.000
Likelihood Ratio	815.681	11	0.000
No. of Valid Cases	655		

[a]14 cells (58.3%) have expected count less than 5. The minimum expected count is 0.31.

All the laboratory samples were swabs, i.e., 100% (206 out of a total number of 206). The highest number of samples were collected from urine in females (100%; 245 out of 245), semen in males (100%; 113 out of 113) and CSF from both genders (100%; 60 out of 60), whereas the remaining patient samples individually made up less than 3.5% of the total number. The statistics show that the difference between patients and laboratory with respect to the type of sample is significant (Chi-Square=0.000). This finding does not agree with an earlier study by La Scolea et al. (1984) in which a total of 2,031 sequential CSF specimens were tested, of which 63 (3.1%) were positive, being derived from cultures from the same number of patients.

Table 3.31 summarizes the classification of bacteria according to growth on the mannitol salt agar (MSA) culture media of patient and laboratory specimens collected at a private laboratory and three hospitals.

Table 3.31: Classification of laboratory and hospital patient MSA cultures

Mannitol Salt Agar (MSA) Culture Classification		Type of Sample		
		Laboratory	Patient	Total
BFMSA	**n**	33	118	151
	%	21.9%	78.1%	100%
BNFMSA	**n**	14	47	61
	%	23.0%	77.0%	100%
GF	**n**	4	0	4
	%	100%	0.0%	100%
GBUND	**n**	2	1	3
	%	66.7%	33.3%	100%
NGB	**n**	118	283	401
	%	29.4%	70.6%	100%
Total	**n**	171	449	620
	%	27.6%	72.4%	100%

BFMSA=bacteria fermentation mannitol salt agar (MSA); BNFMSA=bacteria non-fermentation MSA; GF=growth of fungi; GBUND=Growth Bacteria Undulating *Proteus* (wavy bacteria); NGB=sterile, no growth bacteria

Chi-Square Test	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.615[a]	4	0.002
Likelihood Ratio	16.238	4	0.003
No. of Valid Cases	620		

The highest rate of bacterial fermentation on MSA (BFMSA) was observed in patient samples sourced from hospitals, i.e., 78.1% (118 out of 151 samples) compared with a BFMSA rate of 21.9% (33 out of 151) in samples from a private laboratory. A similar trend was also seen for BNFMSA (bacteria non-fermentation MSA), namely the highest rate in patient samples (77%; 47 out of 61) and for laboratory samples, the BNFMSA was 23% (14 out of 61). All the laboratory samples showed growth of fungi (GF), i.e., 100% (4 out of 4 samples), whereas the growth of wavy bacteria (GBUND; Growth Bacteria Undulating Proteus) was also two-fold higher in laboratory specimens, i.e., 66.7% (2 out of 3) compared to patient samples, i.e., 33.3% (1 out of 3). The rate of NGB (sterile, no growth of bacteria) was greater in hospital specimens, i.e., 70.6% (283 out of 401) compared to an NGB in laboratory specimens of 29.4% (118 out of 401).

Statistical analysis shows that the difference between patients and laboratory with respect to MSA is significant (Chi-Square=0.002). In our study, we found 93% Gram-stain catalase-positive bacteria in laboratory specimens (see Table 3.8 and Figure 3.8). This was almost identical with a study by Grüner et al. (2007), in which katA sequences of *S. aureus* subsp. strains MSSA476, COL, NCTC 8325, USA300, and MW2 were reported. Due to growth of other bacteria, *Streptococcus* and many bacteria were catalase-negative. Moreover, this agrees with a study by Ripolles-Avila et al. (2018) in which bacterial catalase positives were *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. This agrees with a study by Diop et al. (2018) in which gram-positive and catalase-positive bacteria were isolated from samples of patients. In addition, Mukhopadhyay et al. (2017) isolated Gram-positive and catalase-negative cocci from the blood of an 82-year-old male. Moreover, this agrees with a study by Ullah et al. (2018) in which found *S. aureus*, *Saprophyticus* and *E. coli* positive reactions towards catalase. This is congruent with a study by Ripolles-Avila et al. (2018) in which bacterial catalase-positive *S. aureus*, *E. coli* and *P. aeruginosa* were identified as well as with a study by Diop et al. (2018) which found Gram-positive and catalase-positive bacteria in samples of patients.

Table 3.32 shows the diagnostics of bacteria in patient samples from a private laboratory and three hospitals. The highest rate (100%; n=15) was seen for growth of fungi (GF) in the laboratory group of the total specimens tested, while the lowest rate (17.4%, n=161) was for GNB. None of the laboratory specimens tested positive for *Candida* or *Streptococcus*, while patient samples tested 100% positive of the total specimens (n=1 and n=14, respectively). Patient specimens showed the highest rate for GNB (82.6%; 133 out of 161), *S. aureus* (81.6%; 71 out of 87) and NGB, i.e., 59.2% (171 out of 289) tested.

Table 3.32: Diagnostics of bacteria in laboratory and hospital patient samples

Bacterial Diagnostics		Type of Sample		
		Laboratory	Patient	Total
GNB	**n**	28	133	161
	%	17.4%	82.6%	100%
Candida	**n**	0	1	1
	%	0.0%	100%	100%
S. aureus	**n**	16	71	87
	%	18.4%	81.6%	100%
GF	**n**	15	0	15
	%	100%	0.0%	100%
NGB	**n**	118	171	289
	%	40.8%	59.2%	100%
SCN	**n**	28	52	80
	%	35.0%	65.0%	100%
Streptococcus	**n**	0	14	14
	%	0.0%	100%	100%
Total	**n**	205	442	647
	%	31.7%	68.3%	100%

GNB=growth negative bacteria; Candida=*Candida*; GF=growth of fungi; *S. aureus*=Growth of *Staphylococcus* coagulase-positive; NGB=sterile no growth of bacteria; SCN=*Staphylococcus* coagulase-negative; *Streptococcus*.

Chi-Square Test	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	73.172[a]	6	0.000
Likelihood Ratio	81.788	6	0.000
No. of Valid Cases	647		

The statistics show that the difference between patient and laboratory samples with respect to

bacterial diagnostics is significant (Chi-Square=0.000). In the case of GNB (*Enterobacteriaceae*), a total of 647 samples showed significant bacteriuria (82.6%). Our results are agreement with a study by Shakya et al. (2017) which documented 365 (80.9%) *E. coli* and 17 (3.8%) *Klebsiella pneumonia*.

In addition, Balan (2013) detected extended spectrum β lactamases among gram negative clinical isolates, i.e., of 200 samples, 174 yielded organisms belonging to *Enterobacteriaceae* and 26 yielded growth of nonfermenters. Out of 174 members of *Enterobacteriaceae* family, 122 were *E. coli*, 36 *Klebsiella spp*, 8 *Proteus spp*, 5 *Enterobacter spp* and 3 *Citrobacter spp*. Out of 26 nonfermenters, 18 were *Pseudomonas spp* and 8 were *Acinetobacter*.

Moreover, in our study, we found 81.6% *S. aureus*, 65.0% SCN, which agrees with a study by Tadesse et al. (2018) which found a MRSA rate of 68.4%. In laboratory specimens, we found 18.4% *S. aureus* which differs from a study by Sexton et al. (2006) in which the total nosocomial bacteraemia rate was 4.1 cases per 1,000 of hospital patients. Additionally, 25% of patient samples as well as surfaces, air and environment combined were 14 (70%) *Streptococcus*.

We found also *Streptococcus* was 14 (100%) of the total samples which is similar to a study by Ohkoshi et al. (2018), which found *Streptococcus pneumonia* strains from sputum and blood and of patients with disseminated intravascular coagulation in Sapporo City, Japan. About 174 samples yielded organisms belonging to *Enterobacteriaceae*.

Moreover, the study findings agree with a study by Ullah et al. (2018) which reported *S. aureus*-positive reactions towards Gram-staining, coagulase and *Saprophyticus*-positive reaction towards Gram-staining, catalase, and negative reaction towards coagulase and *E. coli*, but showed positive reactions towards catalase.

3.1.6 Comparison of *S. aureus* Resistance and Sensitivity in Laboratory and Hospital Samples

3.1.6.1 *S. aureus* Resistance and Sensitivity to Oxacillin in Laboratory and Hospital Samples

Table 3.33 shows a comparison of *S. aureus* resistance and sensitivity to oxacillin in samples from a private laboratory and three hospitals. The rate for oxacillin-resistant *Staphylococcus aureus* (OXRSA) in patient samples was greater (51.7%; 31 out of 60) than that for laboratory samples (46.2%; 6 out of 13). The highest rate for oxacillin-sensitive *Staphylococcus aureus* (OXSSA) was noted for laboratory samples (53.8%; 7 out of 13) compared with 48.3% (29 out of 60) for patient samples. The statistics show that the difference between patients and laboratory samples with respect to OXRSA and OXSSA is not significant (Chi-Square=0.719).

Table 3.33: Comparison of *S. aureus* resistance and sensitivity to oxacillin in laboratory and hospital samples

Type of Sample: Oxacillin		Type of Sample		
		OXRSA	OXSSA	Total
Laboratory	n	6	7	13
	%	46.2%	53.8%	100%
Patient	n	31	29	60
	%	51.7%	48.3%	100%
Total	n	37	36	73
	%	50.7%	49.3%	100%

OXRSA=oxacillin-resistant *Staphylococcus aureus;* OXSSA=oxacillin-sensitive *Staphylococcus aureus*

Pearson Chi-Square	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
	0.130	1	0.719		
Continuity Correction	0.003	1	0.957		
Likelihood Ratio	0.130	1	0.718		
Fisher's Exact Test				0.768	0.478
No. of Valid Cases	73				

The present investigation found 51.7% MRSA in patient samples which matches a study by Ahmed et al. (2010), in that a MRSA rate of 51% was observed in a total sample size of 170.

This high percentage may be the result of patients that are subjected to various accidents requiring hospitalization (Buzaid et al., 2011). The spread rate of 51.7% in patients may occur from transmission among patients, agreeing with a study that MRSA may be acquired in the hospital environment (French et al., 2004). Moreover, the overuse of β-lactam antibiotics to treat some infections caused by *S. aureus* allows MRSA to propagate in the presence of β-lactam antibiotics, due to a gene that encodes the β-lactam-insensitive penicillin binding protein, PBP2a (Foster, 2018).

The high rate of MRSA in our study could be due to a compromise in the state of aseptic sterilization and disinfection, isolation of MRSA patients and handwashing technique (Maqsood Ali et al., 2007). Our study shows a lower rate of OXRSA in laboratory samples (46.2%). In addition, this agrees with a study by Buzaid et al. (2011) in which they observed a median MRSA range of 10-65% within isolates of *S. aureus*.

This is further strengthened by a recent report by Tadesse et al. (2018) of an OXRSA rate of 68.4%. Our study results are consistent with the 46% OXRSA rate published by Arora et al. (2010). The spread of MRSA may also be caused by hospital staff, as demonstrated by Ahmed et al. (2012), who found 19% MRSA in 569 specimens collected from the anterior nares of the nasal passages of health care workers in Tripoli, Libya.

3.1.6.2 *S. aureus* Resistance and Sensitivity to Vancomycin in Laboratory and Hospital Samples

Table 3.34 displays a comparison of *S. aureus* resistance and sensitivity to vancomycin in samples from a private laboratory and three hospitals. The rate of vancomycin-resistant *Staphylococcus aureus* (VRSA) was 15.4% (2 out of 13) in laboratory specimens vs 1.7% (1 out of 60) in patient specimens. Vancomycin-sensitive *Staphylococcus aureus* (VSSA) detection was high in both laboratory (84.6% (11 out of 13) and patient (98.3%; 59 out of 60) specimens. Our study data agree with those reported by Pandya et al. (2014), namely, that

MRSA was found to be 100% sensitive to vancomycin.

Table 3.34: Comparison of *S. aureus* resistance and sensitivity to vancomycin in laboratory and hospital samples

Type of Sample: Vancomycin		Type of Sample		
		VRSA	VSSA	Total
Laboratory	n	2	11	13
	%	15.4%	84.6%	100%
Patient	n	1	59	60
	%	1.7%	98.3%	100%
Total	n	3	70	73
	%	4.1%	95.9%	100%

VRSA=vancomycin-resistant *Staphylococcus aureus;* VSSA=vancomycin-sensitive *Staphylococcus aureus*

Chi-Square Test	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	13.064[a]	6	0.042	0.136
Likelihood Ratio	9.302	6	0.157	0.074
Fisher's Exact Test	12.924			0.067
No. of Valid Cases	73			

[a]10 cells (71.4%) have expected count less than 5. The minimum expected count is 0.04.

By contrast, our study is inconsistent with that of Arora et al. (2010) in that 46% of the isolated MRSA strains included other strains not resistant to vancomycin. Of the total samples (n=73), our results indicate 4.1% VRSA in laboratory and patient specimens, which does not agree with a study by Pandya et al. (2014) that observed no resistance to vancomycin (MRSA rate was 0%). The ability of bacteria to grow and adapt in laboratory situations for several months (Schut et al. 1993) has stimulated research into MRSA transfer and disease vectors such as flies (Onwugamba et al., 2018). Newer evidence suggest that vancomycin should be used to treat MRSA (Chandak et al., 2018).

3.1.6.3 *S. aureus* Resistance and Sensitivity to Tetracycline in Laboratory and Hospital Samples

Table 3.35 presents a comparison of *S. aureus* resistance and sensitivity to tetracycline in samples from a private laboratory and three hospitals. The proportions of tetracycline-resistant *Staphylococcus aureus* (TRSA) were almost similar in laboratory specimens (46.2%; 6 out of

13) and patient specimens (50%; 30 out of 60), as were the proportions of tetracycline-sensitive *Staphylococcus aureus* (TSSA) in laboratory (53.8%; 7 out of 13) and patient (50%; 30 out of 60) specimens. Thus, the overall rates of TRSA and TSSA in both laboratory and patient samples were evenly matched, i.e., 49.3% (36 out of 73) and 50.7% (37 out of 73), respectively.

The statistics show that the difference between patient and laboratory specimens with respect to TRSA and TSSA is insignificant (Chi-Square=0.801). We found extensive contamination with TRSA in the laboratory and hospital settings. This finding agrees with a study that MRSA may be found in the hospital environment (French et al., 2004). Likewise, Tadesse et al. (2018) found TRSA at a rate of 57%. In addition, our results agree with a study by Pandya et al. (2014) which found TRSA and TSSA at rates of 40.24% and 57.32%, respectively. Tadesse et al. (2018) observed TRSA at a rate of 57%, which does not agree with a study by Naimi et al. (2017) who reported a TSSA rate of 81%.

Table 3.35: Comparison of *S. aureus* resistance and sensitivity to tetracycline in laboratory and hospital samples

Type of Sample: Tetracycline		Type of Sample		
		TRSA	TSSA	Total
Laboratory	n	6	7	13
	%	46.2%	53.8%	100%
Patient	n	30	30	60
	%	50.0%	50.0%	100%
Total	n	36	37	73
	%	49.3%	50.7%	100%

TRSA=tetracycline-resistant *Staphylococcus aureus;* TSSA=tetracycline-sensitive *Staphylococcus aureus*

Pearson Chi-Square	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
	0.063	1	0.801		
Continuity Correction	0.000	1	1.000		
Likelihood Ratio	0.063	1	0.801		
Fisher's Exact Test				1.000	0.522
No. of Valid Cases	73				

3.1.6.4 *S. aureus* Resistance and Sensitivity to Erythromycin in Laboratory and Hospital Samples

Table 3.36 depicts a comparison of *S. aureus* resistance and sensitivity to erythromycin in samples from a private laboratory and three hospitals. None of the laboratory samples showed any evidence of erythromycin-intermediate-sensitive *Staphylococcus aureus* (EISSA), but 1 out of 60 patient samples (1.6%) showed EISSA. Erythromycin-resistant *S. aureus* (ERSA) detection was high in both laboratory (61.5%; 8 out of 13) and patient (76.7%; 46 out of 60) samples, but erythromycin-sensitive *S. aureus* (ESSA) was lower in the aforementioned samples, i.e., 38.5% (5 out of 13) and 21.7%; 13 out of 60), respectively. Overall, the rate of ERSA was high (73.9%; 54 out of 73) and ESSA was moderate (24.66%; 18 out of 73), but EISSA was negligible (1.37%; 1 out of 73). The statistics show that the difference between laboratory and patient samples with respect to ERSA and ESSA is insignificant (Chi-Square=0.413). In our study, we found extensive contamination with ERSA and ESSA in the laboratory which may be transmitted to patients. This agrees with a study by French et al. (2004) in which MRSA was found in the hospital environment.

Table 3.36: Comparison of *S. aureus* resistance and sensitivity to erythromycin in laboratory and hospital samples

Type of Sample: Erythromycin		Type of Sample			
		EISSA	ERSA	ESSA	Total
Laboratory	n	0	8	5	13
	%	0.0%	61.5%	38.5%	100%
Patient	n	1	46	13	60
	%	1.6%	76.7%	21.7%	100%
Total	n	1	54	18	73
	%	1.37%	73.97%	24.66%	100%

EISSA=erythromycin-intermediate-sensitive *Staphylococcus aureus*; ERSA=erythromycin-resistant *S. aureus*; ESSA=erythromycin-sensitive *S. aureus*

Chi-Square Test	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	1.770[a]	2	0.413	0.415
Likelihood Ratio	1.823	2	0.402	0.415
Fisher's Exact Test	2.118			0.415
No. of Valid Cases	73			

[a]3 cells (50.0%) have expected count less than 5. The minimum expected count is 0.18.

Our results are congruent with a study by Tadesse et al. (2018) which detected ERSA at a rate of 51.9%. This was also consistent with recent assertions of an ERSA rate of 79.27% (Furuno et al., 2018; Pandya, 2014), and an ERSA of 73% (Arora et al., 2010). Furthermore, a study by Sexton et al. (2006), suggesting potential ecological pollution. This pointed to ineffective and inaccurate current approaches to cleaning and decontamination methods. The increase in the percentage of pollution in laboratories was due to poor management follow-up, lack of awareness and lack of spending. Such a high spread of MRSA in our study may be due to several factors, including non-compliant use of antibiotics or irregular use without prescription by a doctor, indiscriminate use of antibiotics, lack of realization and unethical treatment before coming to the hospital (Anupurba et al., 2003).

3.1.6.5 *S. aureus* Resistance and Sensitivity to Gentamicin in Laboratory and Hospital Samples

Table 3.37 shows a comparison of *S. aureus* resistance and sensitivity to gentamicin in patient samples from a private laboratory and three hospitals. The gentamicin-intermediate-sensitive *S. aureus* (GISSA) rates in patient samples was 5.0% (3 out of 60), whereas no GISSA was detected in laboratory samples. The rate of gentamicin-resistant *S. aureus* (GRSA) was higher in laboratory samples (30.8%; 4 out of 13) compared to patient samples (11.7%; 7 out of 60). However, the detection of gentamicin-sensitive *S. aureus* (GSSA) strains was greater in patient samples (83.3%; 50 out of 60) compared to laboratory samples (69.2%; 9 out of 13). Overall, the GSSA rate (80.8%; 59 out of 73) was greater than the GRSA rate (15.1%; 11 out of 73), whereas the GISSA rate was relatively low (4.1%; 3 out of 73). The statistics show that the difference between patient and laboratory samples with respect to GRSA, GSSA and GISSA is insignificant (Chi-Square=0.174).

In this study, the sensitivity counts of gentamicin (83.3%) is consistent with studies by Moorhouse et al. (1996) and Furuno et al. (2018) which documented GSSA of 89%. Our study shows higher incidences of GRSA, GSSA and GISSA than those reported by Pandya et al.

(2014), namely, GRSA (37.80%), GSSA (59.76%), GISSA (2.44%). Also, in terms GSSA, of our study was not consistent with that of Arora et al. (2010) which found GSSA to be 73% of the total samples (n=115).

Table 3.37: Comparison of *S. aureus* resistance and sensitivity to gentamicin in laboratory and hospital samples

Type of Sample: Gentamicin		Type of Sample			
		GISSA	GRSA	GSSA	Total
Laboratory	n	0	4	9	13
	%	0.0%	30.8%	69.2%	100%
Patient	n	3	7	50	60
	%	5.0%	11.7%	83.3%	100%
Total	n	3	11	59	73
	%	4.1%	15.1%	80.8%	100%

GISSA=gentamicin-intermediate-sensitive *Staphylococcus aureus*; GRSA=gentamicin-resistant *S. aureus*; GSSA=gentamicin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.500		0.174
Likelihood Ratio	3.579	2	0.167
No. of Valid Cases	73		

3.1.6.6 *S. aureus* Resistance and Sensitivity to Kanamycin in Laboratory and Hospital Samples

Table 3.38 displays a comparison of *S. aureus* resistance and sensitivity to kanamycin in samples from a private laboratory and three hospitals. The rates of kanamycin-resistant *S. aureus* (KRSA) in laboratory and patient specimens were at similar levels, i.e., 53.8% (7 out of 13) and 50% (30 out of 60), respectively. An almost similar trend was observed with kanamycin-sensitive *S. aureus* (KSSA), i.e., 46.2% (6 out of 13) and 50% (30 out of 60), respectively. Overall, KRSA and KSSA levels were also equivalent, i.e., 50.7% (37 out of 73) and 49.3% (36 out of 73), respectively. The statistics show that the difference between patients and laboratory samples with respect to KRSA and KSSA is insignificant (Chi-Square=0.801). The present investigation does not agree with a study by Edslev et al. (2018) which found *S.*

aureus was susceptible to kanamycin. Moreover, our study does not agree with a study by Zriouil et al. (2012) which reported 96.4% MRSA resistance to kanamycin and the work of Kondo et al. (1991) which documented 98% MRSA resistance to kanamycin.

3.1.6.7 *S. aureus* Resistance and Sensitivity to Streptomycin in Laboratory and Hospital Samples

Table 3.39 displays a comparison of *S. aureus* resistance and sensitivity to streptomycin in samples from a private laboratory and three hospitals.

Table 3.38: Comparison of *S. aureus* resistance and sensitivity to kanamycin in laboratory and hospital samples

Type of Sample: Kanamycin		Type of Sample		
		KRSA	KSSA	Total
Laboratory	n	7	6	13
	%	53.8%	46.2%	100%
Patient	n	30	30	60
	%	50.0%	50.0%	100%
Total	n	37	36	73
	%	50.7%	49.3%	100%

KRSA=kanamycin-resistant *S. aureus*; KSSA=kanamycin-sensitive *S. aureus*

Pearson Chi-Square	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
	0.063	1	0.801		
Continuity Correction	0.000	1	1.000		
Likelihood Ratio	0.063	1	0.801		
Fisher's Exact Test				1.000	0.522
No. of Valid Cases	73				

The detection levels for streptomycin-sensitive *S. aureus* (SSSA) in laboratory and patient samples were equivalent, i.e., 84.6% (11 out of 13) vs 81.7% (60 out of 73). Similarly, streptomycin-resistant *S. aureus* (SRSA) in laboratory and patient samples were almost identical, i.e., 15.4% (2 out of 13) vs 18.3% (11 out of 60).

Table 3.39: Comparison of *S. aureus* resistance and sensitivity to streptomycin in laboratory and hospital samples

Type of Sample: Streptomycin		Type of Sample		
		SRSA	SSSA	Total
Laboratory	n	2	11	13
	%	15.4%	84.6%	100%
Patient	n	11	49	60
	%	18.3%	81.7%	100%
Total	n	13	60	73
	%	17.8%	82.2%	100%

SRSA=streptomycin-resistant *S. aureus*; SSSA=streptomycin-sensitive *S. aureus*

Pearson Chi-Square	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
	0.063	1	0.801		
Continuity Correction	0.000	1	1.000		
Likelihood Ratio	0.065	1	0.798		
Fisher's Exact Test				1.000	0.581
No. of Valid Cases	73				

The statistics show that the difference between patients and laboratory samples with respect to SRSA and SSSA is insignificant (Chi-Square=0.581). In our study, the observed SRSA of 18.3% in patient samples does not agree with an SRSA of 55% documented by Okwu et al., (2012) as well as an SRSA of 57.1% observed by Umaru et al. (2017), albeit in isolates of cow's milk.

3.1.6.8 *S. aureus* Resistance and Sensitivity to Amikacin in Laboratory and Hospital Samples

Table 3.40 shows a comparison of *S. aureus* resistance and sensitivity to amikacin in samples from a private laboratory and three hospitals. Amikacin-intermediate-sensitive *S. aureus* (AISSA) and amikacin-resistant *S. aureus* (ARSA) strains were non-detectable in laboratory specimens, whereas amikacin-sensitive *S. aureus* (ASSA) were observed in all laboratory specimens (100%; 13 out of 13). Likewise, ASSA strains were detected in the majority of

patient samples (86.7%; 52 out of 60), ARSA to a much lesser extent (11.7%; 7 out of 60), and AISSA nearly absent (1.37%; 1 out of 60).

Overall, in all samples, the rate of ARSA was moderate (9.6%; 7 out of 73) and ASSA at a 9-fold greater rate (89.0%; 65 out of 73). The statistics show that the difference between laboratory and patient samples with respect to ARSA and ASSA is insignificant (Chi-Square=0.378). Our study findings do not agree with a 28% and 36% resistance to amikacin reported by Durga Suryadevara et al. (2017) and Kondo et al. (1991), respectively.

Table 3.40: Comparison of *S. aureus* resistance and sensitivity to amikacin in laboratory and hospital samples

Type of Sample: Amikacin		Type of Sample			
		AISSA	ARSA	ASSA	Total
Laboratory	n	0	0	13	13
	%	0.0%	0.0%	100%	100%
Patient	n	1	7	52	60
	%	1.7%	11.7%	86.7%	100%
Total	n	1	7	65	73
	%	1.4%	9.6%	89.0%	100%

AISSA=amikacin-intermediate-sensitive *Staphylococcus aureus*; ARSA=amikacin-resistant *S. aureus*; ASSA=amikacin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.947	2	0.378
Likelihood Ratio	3.345	2	0.188
No. of Valid Cases	73		

3.1.6.9 *S. aureus* Resistance and Sensitivity Verified by Polymerase Chain Reaction (PCR)

Table 3.41 shows a comparison of *S. aureus* resistance and sensitivity verified by PCR in samples from a private laboratory and three hospitals. The rate of PCR positive (PCR+) and PCR negative (PCR-) results have been tested in both laboratory and patient samples. The highest rate of PCR+ was observed in the laboratory samples (83.3%; 5 out of 6), while the rate of PCR- in the laboratory samples was 16.7% (1 out of 6). By contrast, the rate of PCR+

was very high in patient samples (96.7%; 29 out of 30) while the PCR- results were about 30-fold lower (3.3%; 1 out of 30).

Overall the rate of PCR+ samples was very high (94.4%; 34 out of 36) compared to PCR- samples (5.6%; 2 out of 36). The statistics show that the difference between laboratory and patient samples with respect to the PCR+ and PCR- MRSA is insignificant (Chi-Square=0.193). The results of the present study showed that the prevalence of traditional methods of disk diffusion and MIC strips, using oxacillin, was efficient in the detection of MRSA because the error of PCR- which accounted for 5.6% agreed with a study by Mohanasoundaram et al. (2008), in which one strain of the MRSA isolates showed as PCR- due to borderline resistance due to hyperproduction of beta-lactamases (Foster, 2018).

Table 3.41: Comparison of *S. aureus* resistance and sensitivity verified by PCR in laboratory and hospital samples

Type of Sample: PCR		Type of Sample		
		PCR-	PCR+	Total
Laboratory	n	1	5	6
	%	16.7%	83.3%	100%
Patient	n	1	29	30
	%	3.3%	96.7%	100%
Total	n	2	34	36
	%	5.6%	94.4%	100%

PCR-=PCR negative, i.e., no *mecA1* and *mecA2* genes detected; PCR+=PCR positive, i.e., *mecA1* and *mecA2* genes detected;

Pearson Chi-Square	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
	1.694	1	0.193		
Continuity Correction	0.106	1	0.745		
Likelihood Ratio	1.273	1	0.259		
Fisher's Exact Test				0.310	0.310
No. of Valid Cases	36				

PCR technology is a reliable method for the determination of MRSA, but it is very expensive. Our results are likewise consistent with those of Chambers (1997), which asserted that the

oxacillin PCR method is the more consistent when compared to the disc diffusion sensitivity method. In addition to S*CCmec* variants, patient samples contain densities of certain MRSA strains which can be identified by PCR (Wolk et al., 2009). In the case of resistant *S. aureus*, only 55% of all MRSA carrying the *mecA gene* tested positive with the disk diffusion method (Kareem, 2013).

Antimicrobial susceptibility testing was conducted by agar disk diffusion where MRSA isolates carried five different *SCCmec* elements; this was the first time mecC-MRSA isolates from animals in Austria (Schauer et al., 2018) were tested for MRSA by four phenotypic methods. Clinical MRSA infection can be detected using the PCR which has 88% sensitivity and 90.1% specificity (Chandak et al., 2018). Also, one should be able to detect MRSA by PCR testing in patients with MRSA pneumonia (Baby et al., 2017).

3.1.7 Comparison of Samples Collected According to Age Group

Table 3.42 shows the numbers and percentages of specimens collected from females and males according to age group distribution. The number of the specimens collected from female patients exceeded that from male patients in the majority of age groups, i.e., 1-9 years (82.4% vs 17.6%), 10-19 years (55.6% vs 44.4%), 20-29 years (81.9% vs 19.1%), except in the 30-49 years age group, the number of specimens was greater than that of female specimens (70.9% vs 21.9%) and for the age group 50-80 years equivalent sample numbers were collected.

Overall, the number of specimens collected from females was also more than collected from males (57.4%; 218 out of 380 vs 42.6%; 162 out of 380). The statistics show that the differences between age groups and gender is significant (Chi-Square=0.000). This finding does not agree with a study by Ullah et al. (2018), in which the highest prevalence of samples was observed in female infants (58.88%) compared to male infants (41.11%).

Table 3.42: Specimens collected from females and males according to age group

Age Group (Years)		Gender		
		Female	Male	Total
1-9	n	42	9	51
	%	82.4%	17.6%	100%
10-19	n	10	8	18
	%	55.6%	44.4%	100%
20-29	n	68	15	83
	%	81.9%	18.1%	100%
30-49	n	44	107	151
	%	29.1%	70.9%	100%
50-80	n	9	9	18
	%	50.0%	50.0%	100%
Newborn (<1 year)	n	45	14	59
	%	76.3%	23.7%	100%
Total	n	218	162	380
	%	57.4%	42.6%	100%

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	91.731	5	0.000
Likelihood Ratio	95.980	5	0.000
No. of Valid Cases	380		

Table 3.43 shows the types and numbers of samples collected according to the different age groups. Blood culture samples were collected from newborns (50%) and the 30-49-year old age group (50%). The majority of CSF (spinal cord fluid) samples derived from newborns (64.2%; 34 out of 53) and 1-9-year olds (17%; 9 out of 53). Similarly, the majority of eye swabs were taken from newborns (66.7%) and 1-9-year olds (33.3%). One lung swab was taken from patient between 10-19 years. Semen samples were collected from 20-29-year old (11.3%; 12 out of 106) and 30-49-year old (85.8%; 91 out of 106) patients. Only 2 sputum samples were collected from the 30-49-year old age group. Swabs from operations derived mainly from newborns (30.8%), 10-19 and 30-49-year old age groups (23.1% each), 1-9-year old age group (15.4%) and the 20-29-year old age group (7.7%).

Table 3.43: Types and numbers of samples collected according to age group

Sample Type		Age Group (Years)						
		1-9	10-19	20-29	30-49	50-80	Newborn*	Total
Blood Culture	n	0	0	0	1	0	1	2
	%	0.0%	0.0%	0.0%	50.0%	0.0%	50.0%	100%
CSF	n	9	2	4	2	2	34	53
	%	17.0%	3.8%	7.5%	3.8%	3.8%	64.2%	100%
Eye Swab	n	1	0	0	0	0	2	3
	%	33.3%	0.0%	0.0%	0.0%	0.0%	66.7%	100%
Lung Swab	n	0	1	0	0	0	0	1
	%	0.0%	100%	0.0%	0.0%	0.0%	0.0%	100%
Semen	n	0	0	12	91	3	0	106
	%	0.0%	0.0%	11.3%	85.8%	2.8%	0.0%	100%
Sputum	n	0	0	0	2	0	0	2
	%	0.0%	0.0%	0.0%	100%	0.0%	0.0%	100%
Swab from Operation	n	2	3	1	3	0	4	13
	%	15.4%	23.1%	7.7%	23.1%	0.0%	30.8%	100%
Throat Swab	n	1	0	0	0	0	0	1
	%	100%	0.0%	0.0%	0.0%	0.0%	0.0%	100%
Urine	n	38	12	65	50	12	18	195
	%	19.5%	6.2%	33.3%	25.6%	6.2%	9.2%	100%
Wound Swab	n	0	0	1	2	1	0	4
	%	0.0%	0.0%	25.0%	50.0%	25.0%	0.0%	100%
Total	n	51	18	83	151	18	59	380
	%	13.4%	4.7%	21.8%	39.7%	4.7%	15.5%	100%

*Newborn=age less than 1 year; CSF=spinal cord fluid

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	294.768	45	0.000
Likelihood Ratio	274.575	45	0.000
No. of Valid Cases	380		

One throat swab was collected from a patient in the 1-9-year old age group. Urine samples were collected from all age groups, but mostly from the 20-29-year old (33.3%), 30-49-year old (25.6%) and 1-9-year old (19.5%) age groups. Wound swabs were taken mainly from the 20-29-year old (25.0%), 30-49-year old (50.0%) and 50-80-year old (25.0%) age groups. The 30-49-year old age group provided the most samples (39.7%), followed by the 20-29-year old

(21.8%) and 1-9-year old (13.4%) age groups. The statistics show that the difference between sample type and age group is significant (Chi-Square=0.000).

3.1.7.1 Comparison of Samples Collected from Hospital Departments According to Age Group

Table 3.44 summarizes data on samples collected from hospital departments according to age groups. Only one sample from a 1-9-year old patient was collected from the intensive care unit (ICU) whereas 60.8% (31 out of 51) and 33.3% (17 out of 51) were collected from the Outpatients and Inpatients departments, respectively.

Table 3.44: Comparison of samples collected from hospital departments according to age group

Age Group (Years)		Department				
		ICU	Inpatients	Newborn	Outpatients	Total
1-9	n	1	17	2	31	51
	%	2.0%	33.3%	3.9%	60.8%	100%
10-19	n	0	4	0	14	18
	%	0.0%	22.2%	0.0%	77.8%	100%
20-29	n	0	32	0	51	83
	%	0.0%	38.6%	0.0%	61.4%	100%
30-49	n	0	20	0	131	151
	%	0.0%	13.2%	0.0%	86.8%	100%
50-80	n	0	2	0	16	18
	%	0.0%	11.1%	0.0%	88.9%	100%
Newborn (<1 year)	n	0	22	22	12	56
	%	0.0%	39.3%	39.3%	21.4%	100%
Total	n	1	97	24	255	377
	%	0.3%	25.7%	6.4%	67.6%	100%

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	168.706	15	0.000
Likelihood Ratio	138.212	15	0.000
No. of Valid Cases	377		

In the 10-19-year old age group, the majority of samples derived from the Outpatients department (60.8%; 14 out of 18) and the Inpatients department (22.2%; 4 out of 18). The same

trends were observed for the 20-20-year old age group, i.e., Outpatients department (61.4%; 51 out of 83) and Inpatients department (38.6%; 32 out of 83) as well as the 30-49-year old age group, i.e., Outpatients department (86.8%; 131 out of 151) and Inpatients department (13.2%; 20 out of 151).

In the case of the 50-80-year old age group, the majority of samples derived from the Outpatients department (88.9%; 16 out of 18), whereas the Outpatients department totalled less (11.1%; 2 out of 18). Essentially all of the newborn samples were collected from the Inpatients department (39.3%; 22 out of 56), neonatal (newborn unit) (39.3%; 22 out of 56) and the Inpatients department (21.4%; 12 out of 56). Overall, the majority of samples were collected from the Outpatients department (67.6%; 255 out of 377) and the Inpatients department (25.7%; 97 out of 377). The statistics show that the difference between age group and department is significant (Chi-Square=0.000). In the present investigation, MRSA (i.e., *S. aureus* resistant to oxacillin) was found all age groups. This agrees with a study by Ahmed et al. (2010), in which 51% MRSA cases were identified among a total of 170 samples. In another a study by Zriouil et al. (2012), 160 *S. aureus* strains were isolated from pathological samples of patients (79 cases) and nasal swabs (81) from different age groups. This high percentage may be the result of patients of different age groups that were subjected to various accidents requiring hospitalization (Buzaid et al., 2011).

The ICU staff may be a major cause of infection. It is generally believed that providing education and infection control would decrease most infection rates (Deniz et al., 2017). Moreover, bacteria, both Gram-positive and Gram-negative, in intensive care units caused infection with high mortality, owing to antibiotic treatment failures. This resulted in the increase as well as spread of antimicrobial resistance to β-lactam antibiotics (MacVane, 2017). In addition, Kumari et al. (2008) reported that MRSA in the intensive care units (ICU) represented 10% of the total of 750 strains of *S. aureus*.

Table 3.45 summarizes blood agar culture (BAC) results for the different age groups in hospital departments. Table 3.45 shows that in the 1-9-year old age group, the blood agar culture scores were BHB (27.5%), BNHB (17.6%), GBUND (2%) and NGB (52.9%; i.e., 27 out of 51 samples). In the 10-19-year old age group, the NGB had the highest score (55.6%), followed by BHB (33.3%) and BNHB (11.1%). In the 20-29-year old age group, the BHB score (45.8%) was about 1.5-fold higher than both the BNHB (25.3%) and NGB (28.9%) scores. The BHB score (62.3%) was highest in the 30-49-year old age group compared with BNHB (14.6%) and NGB (23.2%). In the 50-80-year old age group, the NGB score (55.6%) exceeded both the BHB (33.3%) and BNHB (11.1%) scores.

Table 3.45: Blood agar culture results for the different age groups in hospital departments

Age Group (Years)		Blood Agar Culture (BAC)				
		BHB	BNHB	GBUND	NGB	Total
1-9	n	14	9	1	27	51
	%	27.5%	17.6%	2.0%	52.9%	100%
10-19	n	6	2	0	10	18
	%	33.3%	11.1%	0.0%	55.6%	100%
20-29	n	38	21	0	24	83
	%	45.8%	25.3%	0.0%	28.9%	100%
30-49	n	94	22	0	35	151
	%	62.3%	14.6%	0.0%	23.2%	100%
50-80	n	6	2	0	10	18
	%	33.3%	11.1%	0.0%	55.6%	100%
Newborn (<1 year)	n	11	2	0	46	59
	%	18.6%	3.4%	0.0%	78.0%	100%
Total	n	169	58	1	152	380
	%	44.5%	15.3%	0.3%	40.0%	100%

BHB=Bacteria haemolytic blood; BNHB Bacteria no haemolytic blood; NGB=sterile, no growth of bacteria; GBUND=Growth Bacteria Undulating Proteus (wavy bacteria)

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	80.770	15	0.000
Likelihood Ratio	79.029	15	0.000
No. of Valid Cases	380		

In newborns, the NGB score (78%) was 4-fold higher than the BHB score (18.6%), whereas

the BNHB score was 3.4%. Overall (n=380), the highest scores were observed for BHB (44.5%; 169 out of 380) and NGB (40%; 152 out of 380). The statistics show that the difference between age group and blood agar culture is significant (Chi-Square=0.000). It has been reported that the effects of environmental and local patient characteristics determine the range of microbial infections, i.e., of 28% of *S. aureus* strains from 257 blood cultures, 26% were MRSA, but *E. coli* was the most common cause of community-acquired bacteraemia (Douglas et al., 2004).

Table 3.46 summarizes mannitol salt agar results for the different age groups in hospital departments.

Table 3.46: Mannitol salt agar results for the different age groups in hospital departments

Age Group (Years)		Mannitol Salt Agar (MSA)				
		BFMSA	**BNFMSA**	**GNB**	**NGB**	**Total**
1-9	**n**	11	9	1	30	51
	%	21.6%	17.6%	2.0%	58.8%	100%
10-19	**n**	4	2	0	12	18
	%	22.2%	11.1%	0.0%	66.7%	100%
20-29	**n**	27	7	0	49	83
	%	32.5%	8.4%	0.0%	59.0%	100%
30-49	**n**	51	19	0	81	151
	%	33.8%	12.6%	0.0%	53.6%	100%
50-80	**n**	3	1	0	14	18
	%	16.7%	5.6%	0.0%	77.8%	100%
Newborn (<1 year)	**n**	9	3	0	47	59
	%	15.3%	5.1%	0.0%	79.7%	100%
Total	**n**	105	41	1	233	380
	%	27.6%	10.8%	0.3%	61.3%	100%

BFMSA=bacteria fermentation mannitol salt agar (MSA); BNFMSA=bacteria non-fermentation MSA; GNB=Gram-negative bacteria; NGB=sterile, no growth bacteria

	Value	**Df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	25.186	15	0.048
Likelihood Ratio	23.322	15	0.078
No. of Valid Cases	380		

In the 1-9-year old age group, the NGB score (58.8%) was several times higher than the BFMSA (2.7-fold; 21.6%), BNFMSA (3-fold; 17.6%) and GNB (30-fold; 2.0%). In the 10-19-year old age group, the NGB score (66.7%) was 3-fold higher than the BFMSA (22.2%) and 6-fold higher than the BFMSA (11.1%) scores. Both the NGB (59.0%) and BFMSA (32.5%) scores were high in the 20-29-year old age group. A similar trend was observed in the 30-49-year old age group, i.e., NGB (53.6%) and BFMSA (33.8%).

However, in the 50-80-year old age group and newborn group, the NGB scores were 77.8% and 79.7%, respectively, whereas equivalent BFMSA scores were observed for these groups, i.e., 16.7% and 15.3%, respectively. Overall, the NGB scores were higher than the other scores in all age groups, whereas BFMSA scores were the lowest and GNB was detected in only one sample from the 1-9-year old age group. The statistics show that the difference between age group and mannitol salt agar culture is significant (Chi-Square=0.048).

Table 3.47 summarizes Gram-stain results for the different age groups in hospital departments.

Table 3.47: Gram-stain results for the different age groups in hospital departments

Age Group (Years)		Gram-stain		
		G-	G+	Total
1-9	n	11	18	29
	%	37.9%	62.1%	100%
10-19	n	4	5	9
	%	44.4%	55.6%	100%
20-29	n	30	28	58
	%	51.7%	48.3%	100%
30-49	n	61	57	118
	%	51.7%	48.3%	100%
50-80	n	5	3	8
	%	62.5%	37.5%	100%
Newborn (<1 year)	n	3	11	14
	%	21.4%	78.6%	100%
Total	n	114	122	236
	%	48.3%	51.7%	100%
G+=Gram-positive bacteria; G-=Gram-negative bacteria				

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.813	5	0.235
Likelihood Ratio	7.116	5	0.212
No. of Valid Cases	236		

In the 1-9-year old age group, the Gram-positive (G+) score (62.1%) was 1.6-fold greater than the Gram-negative (G-) score (37.9%). In the 10-19-year old age group, G+ (55.6%) eclipsed the G- (44.4%) counts, whereas in the 20-29 and 30-49-year old age groups, the opposite trend was noted, i.e., G- counts (51.7%) were greater than the G+ counts (48.3%). In the 50-80-year old age group, G- counts (62.5%) were greater than G+ counts (37.5%), but in the newborn group, the opposite leaning occurred, namely, G+ counts (78.6%) exceeded G- counts (21.4%). Overall, the G- and G+ counts were evenly distributed in the total sample (n=236), i.e., G- (48.3%; 114 out of 236) vs G+ (51.7% 122 out of 236). The statistics show that the difference between age group and Gram-stain is not significant (Chi-Square=0.236).

Table 3.48 summarizes catalase results for the different age groups in hospital departments.

Table 3.48: Catalase results for the different age groups in hospital departments

Age Group (Years)		Catalase		
		Negative	**Positive**	**Total**
1-9	**n**	2	23	25
	%	8.0%	92.0%	100%
10-19	**n**	0	9	9
	%	0.0%	100%	100%
20-29	**n**	2	49	51
	%	3.9%	96.1%	100%
30-49	**n**	11	105	116
	%	9.5%	90.5%	100%
50-80	**n**	0	8	8
	%	0.0%	100%	100%
Newborn (<1 year)	**n**	1	12	13
	%	7.7%	92.3%	100%
Total	**n**	16	206	222
	%	7.2%	92.8%	100%

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.070	5	0.689
Likelihood Ratio	4.369	5	0.498
No. of Valid Cases	222		

In all age groups, most of the samples were catalase-positive in excess of 90%. Also, overall the majority (92.8%; 206 out of 222) of samples were catalase-positive, signifying *Staphylococcus aureus* since *Streptococcus* is negative to the test. The statistics show that the difference between age group and catalase is not significant (Chi-Square=0.689).

Table 3.49 summarizes coagulase results for the different age groups in hospital departments.

Table 3.49: Coagulase results for the different age groups in hospital departments

Age Group (Years)		Coagulase		
		Negative	**Positive**	**Total**
1-9	**n**	13	4	17
	%	76.5%	23.5%	100%
10-19	**n**	2	3	5
	%	40.0%	60.0%	100%
20-29	**n**	9	17	26
	%	34.6%	65.4%	100%
30-49	**n**	19	27	46
	%	41.3%	58.7%	100%
50-80	**n**	0	3	3
	%	0.0%	100%	100%
Newborn (<1 year)	**n**	2	8	10
	%	20.0%	80.0%	100%
Total	**n**	45	62	107
	%	42.1%	57.9%	100%

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.046	5	0.023
Likelihood Ratio	14.420	5	0.013
No. of Valid Cases	107		

In the 1-9-year old age group, the majority of samples were coagulase-negative (76%), whereas in the other age groups the majority of samples were coagulase-positive, i.e., 10-19-year old age group (60%), 20-29-year old age group (65.4%), 30-49-year old age group (58.7%), 50-80-year old age group (100%) and newborns (80%). Overall, the number of coagulase-positive (57.9%; 62 out of 107) samples exceeded that of the coagulase-negative (42.1%; 45 out of 107). The statistics show that the difference between age group and coagulase is significant (Chi-Square=0.023).

Table 3.50 summarizes bacterial diagnostic test results for the different age groups in hospital departments. The highest BGN score (41.2%) was observed in the 30-49-year old age group, followed sequentially in rank by the 20-29-year old age group (37.5%), 50-80-year old age group (27.8%), 10-19-year old age group (22.2), 1-9-year old age group (21.6%) and the newborns (5.1%). *Candida* was only detected in one sample from the 1-9-year old age group.

Coagulase-positive *S. aureus* strains were identified in samples from all age groups, but the 1-9-year old age group yielded the lowest score (7.8%). Likewise, NGB was positively demonstrated in samples tested from all age groups in the following descending frequency: newborns (76.3%), 50-80-year old age group (55.6%), 10-19-year old age group (50%), 1-9-year old age group (43.1%), 20-29-year old age group (28.8%) and 30-49-year old age group (21.6%).

Coagulase-negative *S. aureus* strains were identified in samples from all age groups, except in the 50-80-year old age group. *Streptococcus* was detected in low levels in the 20-29 (1.3%), 30-49 (6.1%) and newborn (1.7%) age groups, but not in the 1-9, 10-19 and 50-80-year old age groups. Overall, the combined NGB score (37.7%; 141 out of 374) was the highest followed by BGN (30.5%; 114 out of 374). The statistics show that the difference between age group and bacterial diagnostic test is significant (Chi-Square=0.000). In our study, we found *S. aureus* at an overall rate of 16.6% and BGN at 30.5% of the total samples tested (n=374).

Table 3.50: Bacterial diagnostic test results for the different age groups in hospital departments

Age Group (Years)		Bacterial Diagnostic Test						
		BGN	***Candida***	***S. aureus***	**NGB**	**SCN**	***Streptococcus***	**Total**
1-9	**n**	11	1	4	22	13	0	51
	%	21.6%	2.0%	7.8%	43.1%	25.5%	0.0%	100%
10-19	**n**	4	0	3	9	2	0	18
	%	22.2%	0.0%	16.7%	50.0%	11.1%	0.0%	100%
20-29	**n**	30	0	17	23	9	1	80
	%	37.5%	0.0%	21.3%	28.8%	11.3%	1.3%	100%
30-49	**n**	61	0	27	32	19	9	148
	%	41.2%	0.0%	18.2%	21.6%	12.8%	6.1%	100%
50-80	**n**	5	0	3	10	0	0	18
	%	27.8%	0.0%	16.7%	55.6%	0.0%	0.0%	100%
Newborn (<1 year)	**n**	3	0	8	45	2	1	59
	%	5.1%	0.0%	13.6%	76.3%	3.4%	1.7%	100%
Total	**n**	114	1	62	141	45	11	374
	%	30.5%	0.3%	16.6%	37.7%	12.0%	2.9%	100%

BGN=Bacteria Gram-negative=*Enterobacteriacae+Pseudomonas; S. aureus*=*Staphylococcus* coagulase-positive; NGB=sterile, no growth of bacteria; SCN=*Staphylococcus* coagulase-negative

	Value	**Df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	91.477	25	0.000
Likelihood Ratio	95.668	25	0.000
No. of Valid Cases	374		

This agrees with the study of Richards et al. (1999) in which Gram-negative bacteria score was reported as 64% and that of *S. aureus as* 20%. Pathak et al. (2018) reported *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter spp* in clinical specimens. Our observation of *S. aureus* occurring at levels of 16.6% of the total number (n=374) differ from previous assertions that *S. aureus* strains were isolated from 14 patients (31%) of whom 8 had pneumonia, 2 had bronchial superinfection, 2 had sinusitis, and 2 having been colonized.

All these infected patients made a clinical recovery (Meynard et al., 1996). *Streptococcus* were either absent or present in our 374 samples at very low levels (2.9%). Other studies of infections at three major sites had 68% of all infections as nosocomial pneumonia, 31% as urinary tract infections (UTIs), 23% as primary bloodstream infections (BSIs), while 83% of episodes of nosocomial pneumonia were associated with ventilation (Richards et al., 1999).

In addition, a study by Valenza et al. (2008) found that of the 464 patients studied, 63.3% were infected with *S. aureus*, 50% with *Pseudomonas aeruginosa*, 16.6% with other bacterial strains and 414 had fungal strains. Moreover, a study by Hanberger et al. (2011) reported *Staphylococcus epidermidis* MRSA (5.5%) and MSSA (5.9%), and *Streptococcus pneumoniae* (1.0%). In contrast to our observations, Umashankar et al. (2004) found higher levels of *S. aureus* in the age groups 0-10, 11-20, 20-31, 31-40, 41-50 and ≥51 at respective rates of 53.2%, 18.0%, 12.8%, 6.0%, 5.2% and 4.8%. Table 3.51 summarizes Mueller-Hinton agar results for the different age groups in hospital departments. *S. aureus* was detected in all age groups using the Mueller-Hinton agar assay.

3.1.7.2 Comparison of *S. aureus* Resistance and Sensitivity to Antibiotics in Samples Collected According to Age Group

3.1.7.2.1 *S. aureus* Oxacillin-Resistant and-Sensitive Profiles According to Age Group

Table 3.52 and Figure 3.10 show the comparison of *S. aureus* resistance and sensitivity to oxacillin in samples from a private laboratory and three hospitals according to age group.

Table 3.51: Mueller-Hinton agar results for the different age groups in hospital departments

Age Group (Years)		Mueller-Hinton Agar	
		S. aureus	Total
1-9	n	3	3
	%	100%	100%
10-19	n	3	3
	%	100%	100%
20-29	n	16	16
	%	100%	100%
30-49	n	21	21
	%	100%	100%
50-80	n	3	3
	%	100%	100%
Newborn (<1 year)	n	7	7
	%	100%	100%
Total	n	53	53
	%	100%	100%

Table 3.52: Comparison of *S. aureus* resistance and sensitivity to oxacillin in laboratory and hospital samples according to age group

Age Group (Years)		Oxacillin		
		OXRSA	OXSSA	Total
1-9	n	0	3	3
	%	0.0%	100%	100%
10-19	n	2	1	3
	%	66.7%	33.3%	100%
20-29	n	7	9	16
	%	43.8%	56.3%	100%
30-49	n	13	8	21
	%	61.9%	38.1%	100%
50-80	n	1	2	3
	%	33.3%	66.7%	100%
Newborn (<1 year)	n	5	2	7
	%	71.4%	28.6%	100%
Total	n	28	25	53
	%	52.8%	47.2%	100%

OXRSA=oxacillin-resistant *S. aureus*; OXSSA=oxacillin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.243	5	0.283
Likelihood Ratio	7.450	5	0.189
No. of Valid Cases	53		

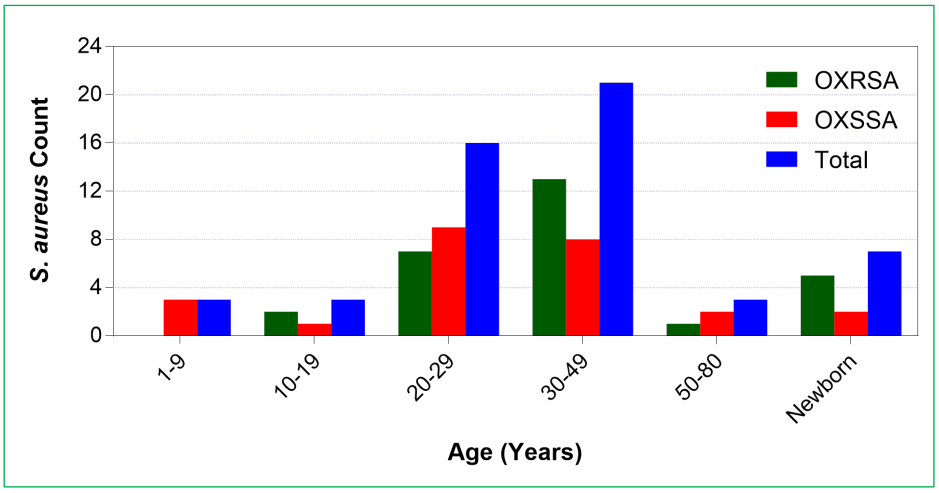


Figure 3.10: *S. aureus* resistance and sensitivity to oxacillin in samples according to age group

The highest rates of oxacillin-resistant *S. aureus* (OXRSA) was found in the newborn age group (71.4%), followed by the 10-19 (66.7%), 30-49 (61.9%), 20-29 (43.8%) and 50-80 (33.3%) year old age groups. The highest rates of oxacillin-sensitive *S. aureus* (OXSSA) was found in the 1-9-year old (100%), 50-80-year old (66.7%) and 20-29-year old (56.3%) age groups. Overall, OXRSA (52.8%) and OXSSA (47.2%) were evenly distributed in the total sample (n=53).

The statistics show that the difference between patient age group and OXRSA and OXSSA is insignificant (Chi-Square=0.283). In the present investigation, MRSA was found in 52.8% in all age groups, which agrees with a study by Ahmed et al. (2010) who found 51% MRSA in 170 samples of *S. aureus* that were resistant to oxacillin, infecting all age categories, except the 1–9-year-old age group. We found MRSA among the 10-19-year old (66.7%), 20-29-year old (43.8%), 30-49-year old (61.9%), 50-80-year old (33.3%) and newborn (71.4%) age groups. This finding was not in agreement with a study by Buzaid et al. (2011) which reported MRSA rates among 1-9-year olds (26.8%), 20-49-year olds (30.9%) and 50-year-olds (36.1%). Moreover, our results were not in agreement with recent studies by Rezaei et al. (2013) and Imani Fooladi et al. (2015) who reported different rates for OXRSA in the isolates of patients. This high percentage of OXRSA may be the result of patients in age groups that are subjected

to various accidents that require their hospitalization (Buzaid et al., 2011). Also using more β-lactam antibiotics to treat some infections caused by *S. aureus* allows MRSA to thrive in the presence of β-lactam antibiotics, due to a gene that encodes the β-lactam-insensitive penicillin-binding protein PBP2a (Foster, 2018). The high percentage of MRSA in our study was presumably the result of a compromise in the state of aseptic sterilization and disinfection, in the isolation of MRSA patients, and in handwashing protocols (Maqsood Ali et al., 2007). We benefited from the diagnosis of MRSA with the aid of oxacillin, in that the results obtained with PCR were much more accurate than the results obtained with oxacillin. The margin with PCR was 0%, while that with oxacillin was 5.4%.

3.1.7.2.2 *S. aureus* Vancomycin-Resistant and-Sensitive Profiles According to Age Group

Table 3.53 and Figure 3.11 show the comparison of *S. aureus* resistance and sensitivity to vancomycin in samples from a private laboratory and three hospitals according to age group.

Table 3.53: Comparison of *S. aureus* resistance and sensitivity to vancomycin in laboratory and hospital samples according to age group

Age Group (Years)		Vancomycin		
		VRSA	VSSA	Total
1-9	n	0	3	3
	%	0.0%	100%	100%
10-19	n	0	3	3
	%	0.0%	100%	100%
20-29	n	0	16	16
	%	0.0%	100%	100%
30-49	n	1	20	21
	%	4.8%	95.2%	100%
50-80	n	0	3	3
	%	0.0%	100%	100%
Newborn (<1 year)	n	0	7	7
	%	0.0%	100%	100%
Total	n	1	52	53
	%	1.9%	98.1%	100%

VRSA=vancomycin-resistant *S. aureus*; VSSA=vancomycin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.553	5	0.907
Likelihood Ratio	1.881	5	0.865
No. of Valid Cases	53		

The only observation of vancomycin-resistant *S. aureus* (VRSA), i.e., 4.8% (1 out of 21) was in the 30–49-year age group, but was absent in the other age groups. For vancomycin sensitive *S. aureus* (VSSA), the rate was 100%. in all age groups, except the 30–49-year age group (95.2%; 20 out of 21). Overall, the VSSA was higher (98.1%; 52 out of 53) than the VRSA (1.9%; 1 out of 53). The statistics show that the difference between VRSA and VSSA and patients' age group is insignificant (Chi-Square=0.907).

Figure 3.11: *S. aureus* resistance and sensitivity to vancomycin in samples according to age group

The high VSSA in the 1–9-, 10–19-, 50–80-, and newborn age groups which amounted to 100% in this study was not in agreement with a study by Ahmed et al. (2012) in Tripoli, Libya, which found the vancomycin sensitivity was 88%. In addition, vancomycin resistance was 27% (Ahmed et al., 2010). In this study, it was found that the highest sensitivity was to the antibiotic vancomycin, which amounted to 98.1% overall. This finding is supported by Leibler et al. (2017) and Hassanzadeh et al. (2013). In the latter, none of the total of 13 samples tested were resistant to vancomycin. Leibler et al. (2017) found that sensitivity to vancomycin was 83.4%

and the proportion of resistance was 16.6%. These parameters approximate those reported by Buzzard et al. (2009) who observed a sensitivity of 82.3% to vancomycin and a resistance of 17.7%, similar to our study. Our results corroborate a study conducted by Prakash et al. (2007) who obtained a 90.4% rate of sensitivity to vancomycin and resistance rate of 9.6%. Several studies have proved that MRSA strains were 100% sensitive to vancomycin (Chambers, 1997; Rajaduraipandi et al., 2006). Another study in Libya has also demonstrated that MRSA has sensitivity to vancomycin and the antibiotic is thus a good and effective treatment to eliminate these bacteria (Ahmed et al., 2010). Our results also vary with the results of Ahmed et al. (2012), in Tripoli, in that strains of MRSA were recorded to show 12% resistance to the antibiotic.

3.1.7.2.3 *S. aureus* Tetracycline-Resistant and-Sensitive Profiles According to Age Group

Table 3.54 and Figure 3.12 show the comparison of *S. aureus* resistance and sensitivity to tetracycline in samples from a private laboratory and three hospitals according to age group.

Table 3.54: Comparison of *S. aureus* resistance and sensitivity to tetracycline in laboratory and hospital samples according to age group

Age Group (Years)		Tetracycline		
		TRSA	TSSA	Total
1-9	n	2	1	3
	%	66.7%	33.3%	100%
10-19	n	1	2	3
	%	33.3%	66.7%	100%
20-29	n	13	3	16
	%	81.3%	18.8%	100%
30-49	n	8	13	21
	%	38.1%	61.9%	100%
50-80	n	1	2	3
	%	33.3%	66.7%	100%
Newborn (<1 year)	n	2	5	7
	%	28.6%	71.4%	100%
Total	n	27	26	53
	%	50.9%	49.1%	100%

TRSA=tetracycline-resistant *S. aureus*; TSSA=tetracycline-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.711	5	0.084
Likelihood Ratio	10.269	5	0.068
No. of Valid Cases	53		

The highest rate of tetracycline-resistance to *S. aureus* (TRSA) was in the age group 20-29-years (81%; 13 out of 16), followed by 1-9-year old (66.7%; 2 out of 3), 30-49-year old (38.1%; 8 out of 21), 50-80-year olds (33.3%; 1 out of 3), while the lowest rate of TRSA was in newborns (28.6%; 2 out of 7). The highest rate of TSSA was in the newborn age group (71.4%; 5 out of 7), followed by the 50-80- and 10-19-year-old (66.7%; 2 out of 3, and 66.7%; 3 out of 3, respectively) and 30-49-year-old (61.9%; 13 out of 21) age groups, while the lowest rate of TSSA was in the 20-29-year old age group (18.8%; 3 out of 16).

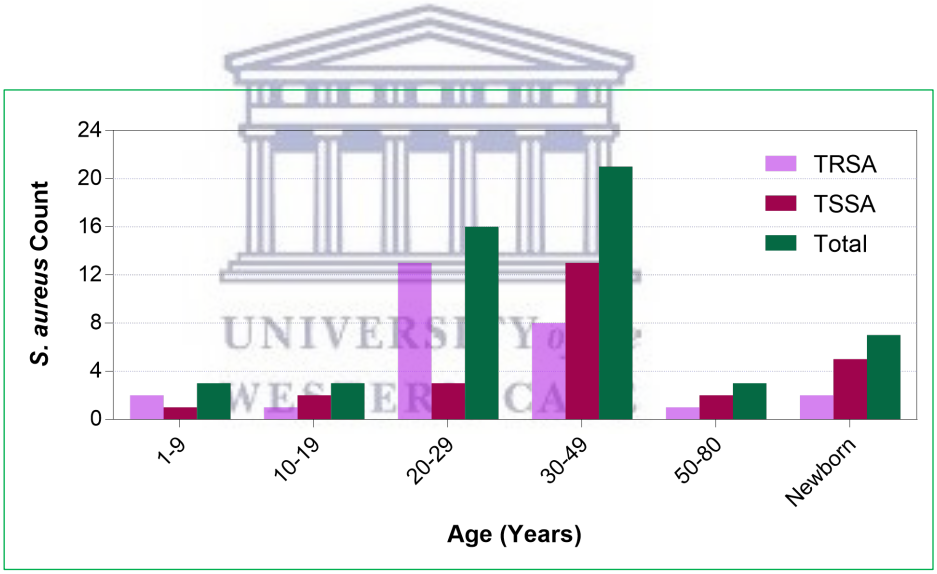


Figure 3.12: *S. aureus* resistance and sensitivity to tetracycline in samples according to age group

The statistics show that the difference between the age group of patients and tetracycline with respect to TRSA and TSSA is insignificant (Chi-Square0.084). Our study does not agree with the results reported by Orrett & Land (2006), namely 78.7% TRSA rates and 73.5% TSSA rates. The count was 28.6% which did not agree with Orrett & Land (2006). In the present investigation, 50.9% TRSA and 49.1% TSSA were observed from all age groups combined (n=53). This was not in agreement with the study by Tiwari et al. (2008) which documented

an 82% TRSA of the total (n=150). Leibler et al. (2017) did not detect any TRSA in a total of 13 samples tested. Our TRSA rate of 81.3% in the 20-29-year old age group together with the TRSA rate of 66.7% in the 1-9-year old age group is consistent with a study by Imani Fooladi et al. (2015) in which resistance to tetracycline of MSSA and MRSA was 33% and 90.4%, respectively from a total number of the 123 patients.

3.1.7.2.4 *S. aureus* Erythromycin-Resistant and-Sensitive Profiles According to Age Group

Table 3.55 and Figure 3.13 show the comparison of *S. aureus* resistance and sensitivity to erythromycin in samples from a private laboratory and three hospitals according to age group.

Table 3.55: Comparison of *S. aureus* resistance and sensitivity to erythromycin in laboratory and hospital samples according to age group

Age Group (Years)		Erythromycin			
		EISSA	ERSA	ESSA	Total
1-9	n	0	2	1	3
	%	0.0%	66.7%	33.3%	100%
10-19	n	0	3	0	3
	%	0.0%	100%	0.0%	100%
20-29	n	0	13	3	16
	%	0.0%	81.3%	18.8%	100%
30-49	n	0	16	5	21
	%	0.0%	76.2%	23.8%	100%
50-80	n	0	2	1	3
	%	0.0%	66.7%	33.3%	100%
Newborn (<1 year)	n	1	6	0	7
	%	14.3%	85.7%	0.0%	100%
Total	n	1	42	10	53
	%	1.9%	79.2%	18.9%	100%

EISSA=erythromycin-intermediate-sensitive *S. aureus*; ERSA=erythromycin-resistant *S. aureus*; ESSA=erythromycin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.748	10	0.463
Likelihood Ratio	8.960	10	0.536
No. of Valid Cases	53		

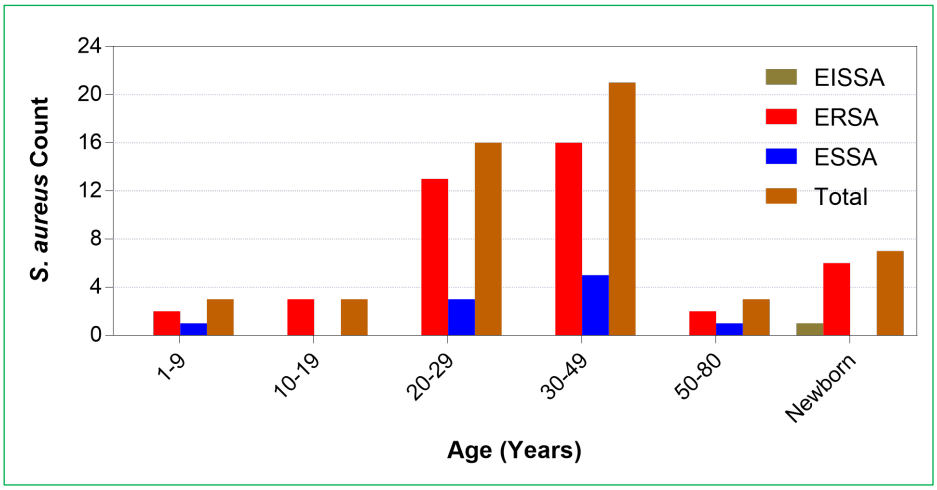


Figure 3.13: *S. aureus* resistance and sensitivity to erythromycin in samples according to age group

Erythromycin-intermediate-sensitive *S. aureus* (EISSA) was detected only in the newborn group (14.3%; 1 out of 7). The highest rate of erythromycin-resistant *S. aureus* (ERSA) was among the age group 10-19-years (100%; 3 out of 3), followed by the 20-29-year old (81.3%; 13 out of 16), 30-49-year old (76.2%; 16 out of 21), 1-9-year old (66.7%; 2 out of 3) and 50-81-year old (66.7%; 2 out of 3) age groups. The highest rate of erythromycin-sensitive *S. aureus* (ESSA) was in the 1-9-year old (33.3%; 1 out of 3) and 50-80-year old (33.3%; 1 out of 3) age groups, whereas in the 30-49-year old age group it was 23.8% (5 out of 21) and in the 20-29-year old age group it was 18.8% (3 out of 16). Overall, the ERSA count was the highest (79.2%; 42 out of 53), while the ESSA count was 18.9% (10 out of 53).

The statistics show that the difference between patients' age group and erythromycin with respect to ERSA, ESSA, EISSA is insignificant (Chi-Square=0.463). In the present investigation, the overall ERSA was high (79.2%, n=53) which agrees with the high ERSA (85%; n=150) reported by Tiwari et al. (2008) as well as the high ERSA (86.7%) documented by Orrett & Land (2006). This is supported by a study by Leibler et al. (2017), in which a total of 13 samples tested for antibiotic resistance to erythromycin showed 81.2% positivity. Our observation is further sustained by Hassanzadeh et al. (2013), who showed, by using PCR, that MRSA resistance to erythromycin occurred at a rate of 58.3%) whereas cited MRSA resistance

to erythromycin tested by PCR were 88.2% (Baby et al., 2017; Ojulong et al., 2009; Smith et al., 2017). Moreover, or results are corroborated by reports of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) rates of 18.2% and 84.6% (n=104), respectively Imani Fooladi et al. (2015).

3.1.7.2.5 *S. aureus* Gentamicin-Resistant and-Sensitive Profiles According to Age Group

Table 3.56 and Figure 3.14 show the comparison of *S. aureus* resistance and sensitivity to gentamicin in samples from a private laboratory and three hospitals according to age group.

Table 3.56: Comparison of *S. aureus* resistance and sensitivity to gentamicin in laboratory and hospital samples according to age group

Age Group (Years)		Gentamicin			
		GISSA	GRSA	GSSA	Total
1-9	n	0	1	2	3
	%	0.0%	33.3%	66.7%	100%
10-19	n	0	0	3	3
	%	0.0%	0.0%	100%	100%
20-29	n	0	1	15	16
	%	0.0%	6.3%	93.8%	100%
30-49	n	2	3	16	21
	%	9.5%	14.3%	76.2%	100%
50-80	n	0	0	3	3
	%	0.0%	0.0%	100%	100%
Newborn (<1 year)	n	1	1	5	7
	%	14.3%	14.3%	71.4%	100%
Total	n	3	6	44	53
	%	5.7%	11.3%	83.0%	100%

GISSA=gentamicin-intermediate-sensitive *S. aureus*; GRSA=gentamicin-resistant *S. aureus*; GSSA=gentamicin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.191	10	0.799
Likelihood Ratio	7.518	10	0.676
No. of Valid Cases	53		

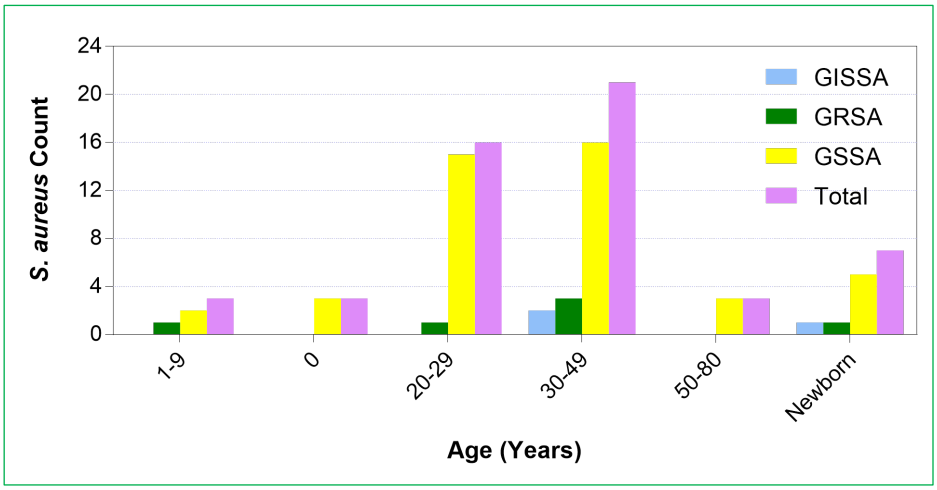


Figure 3.14: *S. aureus* resistance and sensitivity to gentamicin in samples according to age group

Gentamicin-intermediate-sensitive *S. aureus* (GISSA) was detected in the 30-49-year old (9.5%; 2 out of 21) and newborn (14.3%; 1 out of 7) age groups, whereas gentamicin-resistant *S. aureus* (GRSA) was present in the 1-9-year old (33.3%; 1 out of 3), 20-29-year old (6.3%; 1 out of 16), 30-49-year old (14.3%; 3 out of 21) and newborns (14.3%; 1 out of 7), but not in the 10-19-year old and 50-80-year old age groups.

Gentamicin-sensitive *S. aureus* (GSSA) was detected at high levels in all age groups, i.e., 1-9-year old (66.7%; 2 out of 3), 10-19-year old (100%; 3 out of 3), 20-29-year old (93.8%; 15 out of 16), 30-49-year old (76.2%; 16 out of 21), 50-80-year old (100%; 3 out of 3) and newborns (71.4%; 5 out of 7). Overall, the GSSA count was the highest (83%) in all samples (n=53).

The statistics show that the difference between patients' age group and gentamicin with respect to GRSA, GSSA, GISSA is insignificant (Chi-Square=0.799). In the present investigation, GRSA was (11.3%) of all age groups (n=53). This observation is not in agreement with a study by Tiwari et al. (2008) which observed 88% GRSA of the total (n=150). These results signal that vancomycin appears to be the only rational antimicrobial strategy against MRSA (Baby et al., 2017; Ojulong et al., 2009; Smith et al., 2017).

Moreover, we found of 33.3% GRSA in the 1-9-year old age group, which agrees with a study by Seyedi-Marghaki et al. (Seyedi-Marghaki et al., 2019) which found that 71.4% of MRSA strains were resistant to gentamicin. The 33.3% GRSA in the newborn and 1-9 age groups is consistent with a study by Imani Fooladi et al. (2015) which found that resistance of MSSA and MRSA to gentamicin was 6.8% and 48.95%, respectively, out of a total number of 94 patients.

3.1.7.2.6 *S. aureus* Kanamycin-Resistant and-Sensitive Profiles According to Age Group

Table 3.57 and Figure 3.15 show the comparison of *S. aureus* resistance and sensitivity to kanamycin in samples from a private laboratory and three hospitals according to age group.

Table 3.57: Comparison of *S. aureus* resistance and sensitivity to kanamycin in laboratory and hospital samples according to age group

Age Group (Years)		Kanamycin		
		KRSA	KSSA	Total
1-9	n	2	1	3
	%	66.7%	33.3%	100%
10-19	n	2	1	3
	%	66.7%	33.3%	100%
20-29	n	9	7	16
	%	56.3%	43.8%	100%
30-49	n	7	14	21
	%	33.3%	66.7%	100%
50-80	n	1	2	3
	%	33.3%	66.7%	100%
Newborn (<1 year)	n	6	1	7
	%	85.7%	14.3%	100%
Total	n	27	26	53
	%	50.9%	49.1%	100%

KRSA=kanamycin-resistant *S. aureus*; KSSA=kanamycin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.138	5	0.211
Likelihood Ratio	7.592	5	0.180
No. of Valid Cases	53		

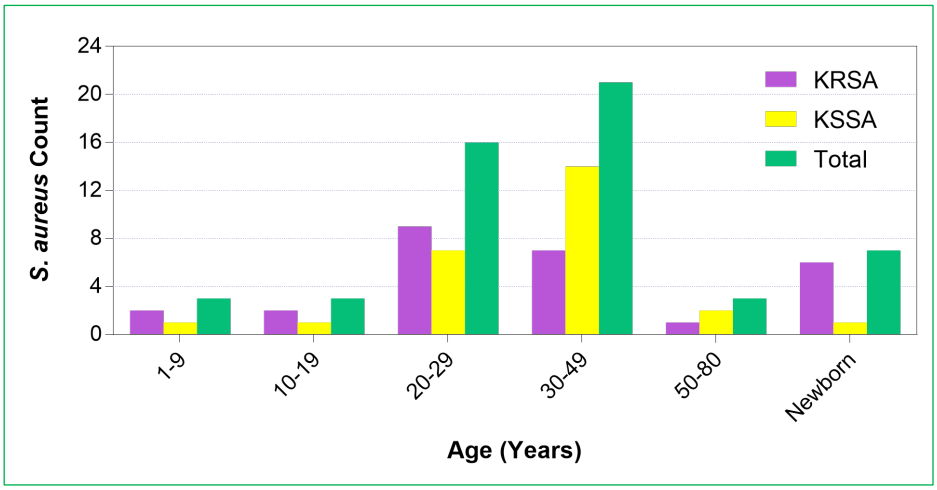


Figure 3.15: *S. aureus* resistance and sensitivity to kanamycin in samples according to age group

The highest rate of kanamycin-resistant *S. aureus* (KRSA) was among the newborn age group (85.7%; 6 out of 7). Both the 1-9-year old and 10-19-year old age groups had a KRSA of 66.7% (2 out of 3), whereas both the 30-49-year old and 50-80-year old age groups had a KRSA of 33.3% (7 out of 21 and 1 out of 3, respectively). The 20-29-year old age group had a KRSA of 56.3% (9 out of 16).

In terms of KSSA, the newborn age group had a low rate (14.3%; 1 out of 7) compared to the rest of the sample, i.e., 33.3% (1 out of 3) for the 1-9-year old and 10-19-year old, 43.8% (7 out of 16) for the 20-29-year old, and 66.7% for the 30-49-year old (14 out of 21) and 50-80-year old (2 out of 3) age groups. Overall, the rates of KRSA and KSSA were evenly distributed, i.e., 50.9% (27 out of 53) and 49.1% (26 out of 53) among the total sample.

The statistics show that the difference between patients' age group and kanamycin with respect to KRSA and KSSA is insignificant (Chi-Square=0.211). In our study, the 50.9%) KRSA of all age groups (of a total n=53) agrees with a study by Edslev et al. (2018) that demonstrated *S. aureus* susceptibility kanamycin. Furthermore, we found an 85.7% KRSA in the newborn age group, which agrees with a study by Seyedi-Marghaki et al. (2019) in which it was found 83% of MRSA strains were resistant to kanamycin. This is also congruent with the finding by

Imani Fooladi et al. (2015) that MSSA and MRSA constituted 33% and 89.4%, respectively, from from the total number (n=122) of patient samples tested.

3.1.7.2.7 *S. aureus* Streptomycin-Resistant and-Sensitive Profiles According to Age Group

Table 3.58 and Figure 3.16 show the comparison of *S. aureus* resistance and sensitivity to streptomycin in samples from a private laboratory and three hospitals according to age group. Streptomycin-resistant *S. aureus* (SRSA) was undetectable in the 1-9-year old, 10-19-year old and 50-80-year old age groups, highest in the 20-29-year old (31.3%; 5 out of 16) and lower in the 30-49-year old (19.0%; 4 out of 21) and newborn (14.3%; 1 out of 7) age groups.

Table 3.58: Comparison of *S. aureus* resistance and sensitivity to streptomycin in laboratory and hospital samples according to age group

Age Group (Years)		Streptomycin		
		SRSA	SSSA	Total
1-9	n	0	3	3
	%	0.0%	100%	100%
10-19	n	0	3	3
	%	0.0%	100%	100%
20-29	n	5	11	16
	%	31.3%	68.8%	100%
30-49	n	4	17	21
	%	19.0%	81.0%	100%
50-80	n	0	3	3
	%	0.0%	100%	100%
Newborn (<1 year)	n	1	6	7
	%	14.3%	85.7%	100%
Total	n	10	43	53
	%	18.9%	81.1%	100%

SRSA=streptomycin-resistant *S. aureus*; SSSA=streptomycin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.138	5	0.211
Likelihood Ratio	7.592	5	0.180
No. of Valid Cases	53		

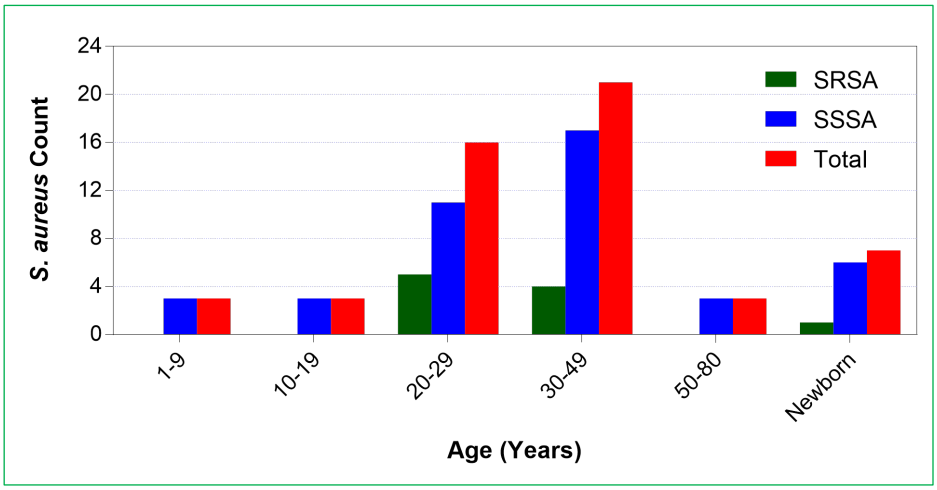


Figure 3.16: *S. aureus* resistance and sensitivity to streptomycin in samples according to age group

In the case of streptomycin-sensitive *S. aureus* (SSSA), high rates were detected in all age groups, i.e., 1-9-, 10-19- and 50-80-year old age groups all scored 100% (3 out of 3 in all cases), followed by the newborn (85.7%; 6 out of 7), 30-49-year old (81.0%; 17 out of 21) and 20-29-year old (68.8%; 11 out of 16) age groups. Overall, the SSSA rate (81.1%; 43 out of 53) also exceeded the SRSA rate (18.9%; 10 out of 53). The statistics show that the difference between patients' age group and streptomycin with respect to SRSA and SSSA is insignificant (Chi-Square=0.211).

3.1.7.2.8 *S. aureus* Amikacin-Resistant and-Sensitive Profiles According to Age Group

Table 3.59 and Figure 3.17 show the comparison of *S. aureus* resistance and sensitivity to amikacin in samples from a private laboratory and three hospitals according to age group. Levels of amikacin-intermediate-sensitive *S. aureus* (AISSA) was undetectable in the 1-9-year old, 10-19-year old, 20-29-year old, 50-80-year old and newborn age groups, but a low level was observed in the 30-49-year old age group (4.8%; 1 out of 21). Amikacin-resistant *S. aureus* (ARSA) was also absent in samples derived from 1-9-year old, 10-19-year old and 50-80-year old age groups, but could be detected in the newborn (42.9%; 3 out of 7), 20-29-year old (12.5%; 2 out of 16) and 30-49-year old (4.8%; 1 out of 21) age groups.

Amikacin-sensitive *S. aureus* (ASSA) was detected in all samples of the 1-9-year old, 10-19-year old and 50-80-year old (100%; 3 out of 3, respectively) age groups, but also in high levels in the 30-49-year old (90.5%; 19 out of 21), 20-29-year old (87.5%; 14 out of 16) and newborn (57.1%; 4 out of 7) age groups. Overall, ASSA levels (86.8%; 46 out of 53) were also greater than those for ARSA (11.3%; 6 out of 53).

The statistics show that the difference between patients' age group and amikacin with respect to ARSA, ASSA and AISSA is insignificant (Chi-Square=0.403). In the present investigation the overall 11.3% ARSA of all age groups (n=53) does not agree with the 80% ARSA (n=150) reported by Mohanasoundaram et al. (2008).

Table 3.59: Comparison of *S. aureus* resistance and sensitivity to amikacin in laboratory and hospital samples according to age group

Age Group (Years)		Amikacin			
		AISSA	ARSA	ASSA	Total
1-9	n	0	0	3	3
	%	0.0%	0.0%	100%	100%
10-19	n	0	0	3	3
	%	0.0%	0.0%	100%	100%
20-29	n	0	2	14	16
	%	0.0%	12.5%	87.5%	100%
30-49	n	1	1	19	21
	%	4.8%	4.8%	90.5%	100%
50-80	n	0	0	3	3
	%	0.0%	0.0%	100%	100%
Newborn (<1 year)	n	0	3	4	7
	%	0.0%	42.9%	57.1%	100%
Total	n	1	6	46	53
	%	1.9%	11.3%	86.8%	100%

AISSA=amikacin-intermediate-sensitive *S. aureus*; ARSA=amikacin-resistant *S. aureus*; ASSA=amikacin-sensitive *S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.434	10	0.403
Likelihood Ratio	9.516	10	0.484
No. of Valid Cases	53		

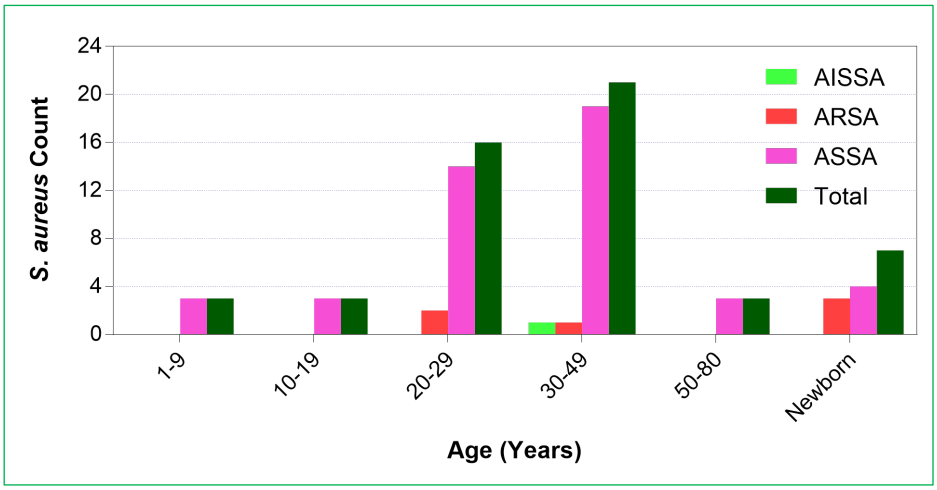


Figure 3.17: *S. aureus* resistance and sensitivity to amikacin in samples according to age group

Our ASSA values of 100% also agree with a study by Edslev et al. (2018). Moreover, our observed ARSA level 42.9% in the newborn group is consistent with a reported ARSA of 59.5% (Seyedi-Marghaki et al., 2019).

3.1.7.2.9 PCR Profiles of *S. aureus*-Resistance and-Sensitivity According to Age Group

Table 3.60 and Figure 3.18 show the PCR analysis of *S. aureus* resistance and sensitivity in samples from a private laboratory and three hospitals according to age group. *S. aureus*-positive strains (PCR+) were identified in all (100%) of samples derived from the 10-19-, 30-49-, 50-80- and newborn age groups, but only in 85.7% of 20-29-year old age group, whereas PCR- strains were identified in the 20-29-year old age group. Overall, most of the samples tested PCR+ for *S. aureus* (96.3%; 26 out of 27) and 3.7% (1 out of 27) tested PCR-.

The statistics show that the difference between patients' age group and PCR is insignificant (Chi-Square=0.563). The results of the present study showed that the prevalence of traditional methods of disk diffusion and MIC strips, using the antibiotic oxacillin was efficient in the detection of MRSA, because of the error of PCR-, which accounted for 3.7%, agreed with the study by Mohanasoundaram et al. (2008) which found one strain of the MSSA isolates negative

to PCR. This was due to borderline resistance owing to hyperproduction of beta-lactamases (Foster, 2018).

Table 3.60: PCR analysis of *S. aureus* resistance and sensitivity in laboratory and hospital samples according to age group

Age Group (Years)		Polymerase Chain Reaction (PCR)		
		PCR-	PCR+	Total
1-9	n	ND	ND	ND
	%	ND	ND	ND
10-19	n	0	2	2
	%	0.0%	100%	100%
20-29	n	1	6	7
	%	14.3%	85.7%	100%
30-49	n	0	12	12
	%	0.0%	100%	100%
50-80	n	0	1	1
	%	0.0%	100%	100%
Newborn (<1 year)	n	0	5	5
	%	0.0%	100%	100%
Total	n	1	26	27
	%	3.7%	96.3%	100%
PCR-=PCR negative; PCR+=PCR positive; ND=not determined				

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.967	4	0.563
Likelihood Ratio	2.813	4	0.590
No. of Valid Cases	27		

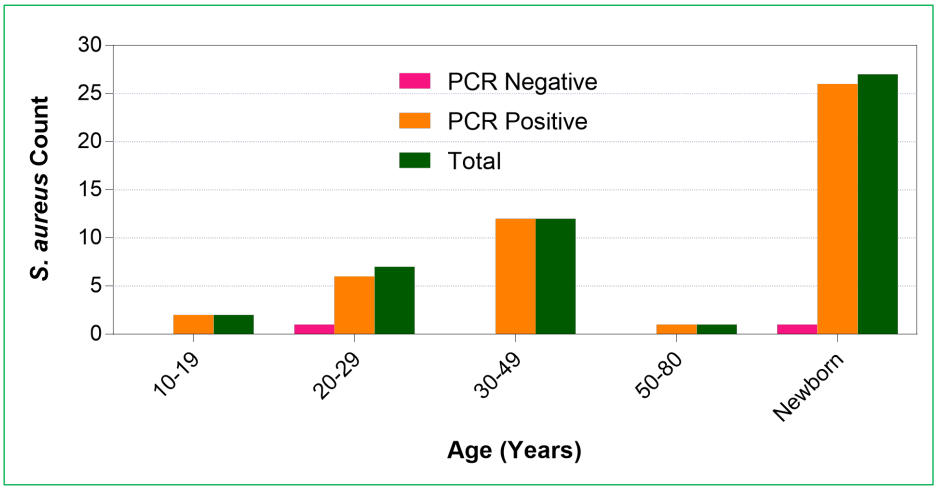


Figure 3.18: PCR analysis of *S. aureus* resistance and sensitivity in samples according to age group

PCR technology is a good measure for the determination of MRSA, but it is very expensive. Our results are consistent with that of Chambers (1997), whereby the oxacillin PCR method is the more reliable when compared to the disc diffusion sensitivity method. In addition to *SCCmec* variants, PCR-positive culture-negative results, as well as patients' samples contain densities of MRSA, which can be identified by PCR (Wolk et al., 2009).

In the case of resistant *S. aureus*, only 55% of all MRSA tested with the disk diffusion method carry the *mecA* gene (Kareem, 2013). Antimicrobial susceptibility testing was conducted by agar disk diffusion, where MRSA isolates carried five different *SCCmec* elements. This was the first time mecC-MRSA was isolated from animals in Austria (Schauer et al., 2018) using genomic and phenotypic methods encompassing an oxacillin resistant screen with mecA-PCR as the gold standard. A total of 103 out of 200 (51.5%) bacterial isolates were *S. aureus* (Thiruvannamalai, et al., 2018). The *mecA* gene was found to be responsible for methicillin resistance in most of the strains isolated (Bhowmick et al., 2018). MRSA is associated with high rates of infection and mortality (Chandak et al., 2018; Sit et al., 2018).

3.1.8 Comparison of Patient Samples Collected at Different Locations

3.1.8.1 Patient Samples Collected at Different Locations According to Age Group

Table 3.61 shows the numbers and percentages of patient samples collected at different locations according to age group. The statistics show that the difference between patients' age group and location is significant (Chi-Square=0.000). At AIH, the majority of patient samples were collected from the 30-49-year old age group (58.9%; 89 out of 151). At CHM, patient samples were collected in the following descending order of magnitude: newborns (100%; 59 out of 59), 1-9-year old (90.2%; 46 out of 51), 10-19-year old (83.3%; 15 out of 18), 20-29-year old (57.8%; 48 out of 83) and 50-80-year old (55.6%; 10 out of 18) age groups. At MCL and NCIM, fewer samples were collected for almost all ages, and overall the total samples collected from these two sites were 9.5% (36 out of 380) and 7.1% (27 out of 380), compared

with the overall numbers collected at AIH (27.1%; 103 out of 380) and 56.3% (214 out of 380), respectively.

Table 3.61: Patient samples collected at different locations according to age group

Age Group (Years)		Location				
		AIH	CHM	MCL	NCIM	Total
1-9	n	0	46	3	2	51
	%	0.0%	90.2%	5.9%	3.9%	100%
10-19	n	0	15	0	3	18
	%	0.0%	83.3%	0.0%	16.7%	100%
20-29	n	12	48	17	6	83
	%	14.5%	57.8%	20.5%	7.2%	100%
30-49	n	89	36	14	12	151
	%	58.9%	23.8%	9.3%	7.9%	100%
50-80	n	2	10	2	4	18
	%	11.1%	55.6%	11.1%	22.2%	100%
Newborn (<1 year)	n	0	59	0	0	59
	%	0.0%	100%	0.0%	0.0%	100%
Total	n	103	214	36	27	380
	%	27.1%	56.3%	9.5%	7.1%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; CIM=National Cancer Institute Misurata.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	190.26	15	0.000
Likelihood Ratio	220.996	15	0.000
No. of Valid Cases	380		

3.1.8.2 Patient Samples Collected at Different Locations According to Gender

Table 3.62 shows the numbers and percentages of patient samples collected at different locations according to gender. All the samples collected from AIH were from females (n=110), which constituted 24.9% of the total samples collected from all sites (110 out of 442). At CHM, samples collected from male patients (n=205) represented 80.1% of the total samples collected from males at all sites (i.e., 205 out of 256), whereas the number of samples collected from female patients (n=59), formed 31.7% of the total sample size for females (i.e., 59 out of 186). At MCL and NCIM, samples collected from male patients were twice that collected from

female patients, i.e., 11.3% (29 out of 256) vs 4.8% (9 out of 186) and 8.6% (22 out of 256) vs 4.3% (8 out of 186), respectively. Overall, the majority of samples collected derived from AIH (24.9%; 110 out of 442) and CHM (59.7%; 264 out of 442). The statistics show that the difference between patients' gender and location is significant (Chi-Square=0.000).

Table 3.62: Patient samples collected at different locations according to gender

Gender		Location				
		AIH	CHM	MCL	NCIM	Total
Male	n	0	205	29	22	256
	%	0.0%	80.1%	11.3%	8.6%	100%
Female	n	110	59	9	8	186
	%	59.1%	31.7%	4.8%	4.3%	100%
Total	n	110	264	38	30	442
	%	24.9%	59.7%	8.6%	6.8%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	856.878	33	0.000
Likelihood Ratio	776.113	33	0.000
No. of Valid Cases	655		

3.1.8.3 Patient Samples Collected at Different Locations According to Specimen Type

Table 3.63 depicts the numbers and percentages of patient samples collected at different locations according to specimen type. Semen samples were collected exclusivey from AIH (97.3%; 110 out of 113) and CHM (2.7%; 3 out of 113). All of the following specimens were collected from CHM: blood culture (100%; 2 out of 2), CSF (100%; 60 out of 60), eye swabs (100%; 3 out of 3), stool sample (100%; 1 out of 1), throat swab (100%; 1 out of 1), whereas swabs (52.9%; 109 out of 206), swabs from operation (87.5%; 14 out of 16) and urine (75.1%; 184 out of 245) represented the remainder of specimens collected from this location. Urine was the only specimen type collected from MCL (16.7%; 41 out of 245). Specimens such as a lung swab (100%; 1 out of 1), sputum (100%; 2 out of 2), swabs (47.1%; 97 out of 206), swabs from operation (12.5%; 2 out of 16), urine (8.2%; 20 out of 245) and wound swabs (100%; 5 out of

5) were obtained from the NCIM. Overall, more than half of the total number of specimens were acquired from CHM (57.6%; 377 out of 655). The statistics show that the difference between specimen type and location is significant (Chi-Square=0.000).

Table 3.63: Patient samples collected at different locations according to specimen type

Specimen Type		Location				
		AIH	CHM	MCL	NCIM	Total
Blood Culture	n	0	2	0	0	2
	%	0.0%	100%	0.0%	0.0%	100%
CSF	n	0	60	0	0	60
	%	0.0%	100%	0.0%	0.0%	100%
Eye Swab	n	0	3	0	0	3
	%	0.0%	100%	0.0%	0.0%	100%
Lung Swab	n	0	0	0	1	1
	%	0.0%	0.0%	0.0%	100%	100%
Semen	n	110	3	0	0	113
		97.3%	2.7%	0.0%	0.0%	100%
Sputum	n	0	0	0	2	2
		0.0%	0.0%	0.0%	100%	100%
Stool	n	0	1	0	0	1
		0.0%	100%	0.0%	0.0%	100%
Swab	n	0	109	0	97	206
		0.0%	52.9%	0.0%	47.1%	100%
Swab from Operation	n	0	14	0	2	16
		0.0%	87.5%	0.0%	12.5%	100%
Throat Swab	n	0	1	0	0	1
		0.0%	100%	0.0%	0.0%	100%
Urine	n	0	184	41	20	245
		0.0%	75.1%	16.7%	8.2%	100%
Wound Swab	n	0	0	0	5	5
		0.0%	0.0%	0.0%	100%	100%
Total	n	110	377	41	127	655
	%	16.8%	57.6%	6.3%	19.4%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	856.878	33	0.000
Likelihood Ratio	776.3	33	0.000
No. of Valid Cases	655		

3.1.8.4 Blood Agar Culture Growth of Patient Samples Collected at Different Locations

Table 3.64 shows the numbers and percentages of blood agar culture growth of patient samples collected at different locations, i.e., three hospitals and one private laboratory. The statistics show that the difference between blood culture and location is significant (Chi-Square=0.000). The highest rate of BHB occurred in specimens collected from the CHM (41.5%; 9 out of 236), while the lowest rate of BHB was observed for specimens from MCL (5.9%; 14 out of 236). The highest rate of BNHB was from CHM (47.7%; 41 out of 86). Likewise, the highest rate of FSCN was from the CHM (78.9%; 15 out of 19).

Table 3.64: Blood agar culture growth of patient samples collected at different locations

Blood Agar Culture (BAC) Growth		Location				
		AIH	CHM	MCL	NCIM	Total
BHB	n	94	98	14	30	236
	%	39.8%	41.5%	5.9%	12.7%	100%
BNHB	n	14	41	16	15	86
	%	16.3%	47.7%	18.6%	17.4%	100%
FSCN	n	0	15	0	4	19
	%	0.0%	78.9%	0.0%	21.1%	100%
GBUND	n	0	10	0	2	12
		0.0%	83.3%	0.0%	16.7%	100%
NGB	n	2	214	11	77	304
		0.7%	70.4%	3.6%	25.3%	100%
Total	n	110	378	41	128	657
	%	16.7%	57.5%	6.2%	19.5%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata; BHB=bacteria haemolytic blood; BNHB=bacteria no haemolytic blood; FSCN=Fungi, *Staphylococcus*-coagulase negative; GBUND=growth bacteria undulating (proteus); NGB=no growth bacteria

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	188.451	12	0.000
Likelihood Ratio	206.138	12	0.000
No. of Valid Cases	657		

The highest rate of GBUNd was from the CHM (83.3%; 10 out of 12), while the lowest rate of GBUNd was from NCIM (16.7%; 2 out of 12), but GBUNd was absent from AIH and MCL. The highest rate of NGB was from the CHM (70.4%; 214 out of 304), followed by NCIM (25.3%; 77 out of 304), while the lowest rate of NGB was from AIH (0.7%; 2 out of 304). Overall, CHM represented more than half of the specimens (57.5%; 378 out of 657) for which bacterial growth was positive in blood agar culture (BAC).

3.1.8.5 Mannitol Salt Agar Culture Growth of Patient Samples Collected at Different Locations

Table 3.65 shows the numbers and percentages of mannitol salt agar culture growth of patient samples collected at different locations, i.e., three hospitals and one private laboratory.

Table 3.65: Mannitol salt agar culture growth of patient samples collected at different locations

Mannitol Salt Agar (MSA) Culture Growth		Location				
		AIH	CHM	MCL	NCIM	Total
BFMSA	n	43	75	8	25	151
	%	28.5%	49.7%	5.3%	16.6%	100%
BNFMSA	n	15	30	2	14	61
	%	24.6%	49.2%	3.3%	23.0%	100%
GFSCN	n	0	0	0	4	4
	%	0.0%	0.0%	0.0%	100%	100%
GNB	n	0	1	0	2	3
		0.0%	33.3%	0.0%	66.7%	100%
NGB	n	52	235	31	83	401
		13.0%	58.6%	7.7%	20.7%	100%
Total	n	110	341	41	128	620
	%	17.7%	55.0%	6.6%	20.6%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata; BFMSA=bacteria fermentation mannitol salt agar; BNFMSA=bacteria non-fermentation mannitol salt agar; GFSCN=fungi, *Staphylococcus*-coagulase-negative; GNB=*Enterobacteraciae*, *Pseudomonas;* NGB=no growth bacteria

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	41.673	12	0.000
Likelihood Ratio	37.699	12	0.000
No. of Valid Cases	620		

The statistics show that the difference between mannitol salt agar (MSA) and location is

significant (Chi-Square=0.000). The highest rates of BFMSA (49.7%; 75 out of 151), BNFMSA (49.7%; 30 out of 61) and NGB (58.6%; 235 out of 401) were observed in samples obtained from CHM. Overall, the growth of bacteria and fungi in MSA was also highest (55.0%; 341 out of 641) in samples from CHM. GFSCN was not observed in samples from AIH, CHM and MCL, but positive in all samples from NCIM (100%; 4 out of 4), while GNB was observed in samples from CHM (33.3%; 1 out of 3) and NCIM (66.7%; 2 out of 3) only.

3.1.8.6 Gram-stain Culture Growth of Patient Samples Collected at Different Locations

Table 3.66 summarizes data of Gram-stain culture growth of patient samples collected at different locations, i.e., three hospitals and one private laboratory.

Table 3.66: Gram-stain culture growth of patient samples collected at different locations

Gram-stain Culture Growth		Location				
		AIH	CHM	MCL	NCIM	Total
G-	n	60	60	20	21	161
	%	37.3%	37.3%	12.4%	13.0%	100%
G+	n	46	105	10	34	195
		23.6%	53.8%	5.1%	17.4%	100%
Total	n	106	165	30	55	356
	%	29.8%	46.3%	8.4%	15.4%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata; G-=Gram-negative bacteria; G+=Gram-positive bacteria

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.440	3	0.001
Likelihood Ratio	17.532	3	0.001
No. of Valid Cases	356		

The rate of Gram-negative bacteria (G-) was equally observed in samples sourced from AIH and CHM (37.3%; 60 out of 161) as well as NCIM (13.0%; 21 out of 161) and MCL (12.4%; 20 out of 161). The rate of Gram-positive bacteria (G+) occurred at high frequency in samples from CHM, followed by AIH (23.6%; 46 out of 195), NCIM (17.4%; 34 out of 195), but at low levels in samples from MCL (5.1%; 10 out of 195). The statistics show that the difference

between Gram-stain culture growth and location is significant (Chi-Square=0.001).

3.1.8.7 Catalase Test Results for Patient Samples Collected at Different Locations

Table 3.67 show catalase test results of patient samples collected at different locations, i.e., three hospitals and one private laboratory. Catalase-negative test results were higher in samples collected from AIH (52.4%; 11 out of 21) and CHM (42.9%; 9 out of 21) compared to MCL (4.8%; 1 out of 21), but undetected in samples from NCIM. On the other hand, catalase-positive test results were higher in CHM (43.2%; 130 out of 301) and AIH (32.2%; 97 out of 301), followed by moderate rates in samples from NCIM (15%; 45 out of 301) and MCL (9.6%; 29 out of 301). CHM samples represented the highest combined overall rates for catalse-negative and-positive samples (43.2%; 139 out of 322), followed by AIH (33.5%; 108 out of 322). The statistics show that the difference between catalase test results and location is not significant (Chi-Square=0.111).

Table 3.67: Catalase test results for patient samples collected at different locations

Catalase Test		Location				
		AIH	CHM	MCL	NCIM	Total
Catalase-	n	11	9	1	0	21
	%	52.4%	42.9%	4.8%	0.0%	100%
Catalase+	n	97	130	29	45	301
		32.2%	43.2%	9.6%	15.0%	100%
Total	n	108	139	30	45	322
	%	33.5%	43.2%	9.3%	14.0%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.018	3	0.111
Likelihood Ratio	8.725	3	0.033
No. of Valid Cases	322		

However, morphologically similar *Enterococcus* or *Streptococcus* (catalase-negative) and *Staphylococcus* (catalase-positive) can be differentiated using the catalase test. We benefited

from the catalase test owing to its robustness in differentiating between *Staphylococcus* and *Streptococcus*, due to *Streptococcus* being negative to the test.

3.1.8.8 Coagulase Test Results for Patient Samples Collected at Different Locations

Table 3.68 show coagulase test results of patient samples collected at different locations, i.e., three hospitals and one private laboratory.

Table 3.68: Coagulase test results for patient samples collected at different locations

Coagulase Test		Location				
		AIH	CHM	MCL	NCIM	Total
Coagulase-	n	14	43	7	23	87
	%	16.1%	49.4%	8.0%	26.4%	100%
Coagulase+	n	20	52	2	10	84
		23.8%	61.9%	2.4%	11.9%	100%
Total	n	34	95	9	33	171
	%	19.9%	55.6%	5.3%	19.3%	100%

AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata; *Streptococcus*=coagulase-negative=SCN; *Staphylococcus* coagulase-positive=*S. aureus*

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.761[a]	3	0.021
Likelihood Ratio	10.070	3	0.018
No. of Valid Cases	171		

[a]2 cells (25.0%) have expected count less than 5. The minimum expected count is 4.42

Statistical analysis shows that the difference between *Staphylococcus* coagulase-positive and *Staphylococcus* coagulase-negative relative to location is significant (Chi-Square=0.021). The highest rates *Staphylococcus* coagulase-negative results were observed in samples fom CHM (49.4%; 43 out of 87), while the lowest rates of *Staphylococcus* coagulase-negative results were found in samples from MCL (8.0%; 7 out of 87). The highest *Staphylococcus* coagulase-positive tests occurred in samples from AIH (23.8%; 20 out of 84) and CHM (61.9%; 52 out of 84). Samples from CHM contributed more than half of the coagulase-negative and-postive test results (55.6%; 95 out of 171), while AIH (19.9%; 34 out of 171) and NCIM (19.3%; 33 out of 171) contributed equally.

3.1.8.9 Comparison of *S. aureus* Resistance and Sensitivity to Antibiotics in Samples According to Location

3.1.8.9.1 *S. aureus* Oxacillin-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.69 and Figure 3.18 show the comparison of *S. aureus* resistance and sensitivity to oxacillin in samples according to location, i.e., a private laboratory and three hospitals.

Table 3.69: Comparison of *S. aureus* resistance and sensitivity to oxacillin in samples according to location

Location		Oxacillin		
		OXRSA	OXSSA	Total
AIH	n	11	4	15
	%	73.3%	26.7%	100%
CHM	n	23	25	48
	%	47.9%	52.1%	100%
MCL	n	1	0	1
	%	100%	0.0%	100%
NCIM	n	2	7	9
	%	22.2%	77.8%	100%
Total	n	37	36	73
	%	50.7%	49.3%	100%

OXRSA=oxacillin-resistant *S. aureus*; OXSSA=oxacillin-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.115	3	0.068
Likelihood Ratio	7.795	3	0.050
No. of Valid Cases	73		

The majority of the samples collected from AIH were OXRSA (73.3%; 11 out of 15) compared with OXSSA (26.7%; 4 out of 15). In the case of samples collected from CHM and NCIM, the rate of OXSSA exceeded that of OXRSA, i.e., 52.1% (25 out of 48) and 77.8% (7 out of 9), respectively. One sample from MCL tested positive for OXRSA (100%; 1 out of 1), but no OXSSA was detected. However, 7 samples from NCIM tested positive for OXRSA (22.2%; 2

out of 9), while 2 samples from the latter location showed an OXRSA rate of 22.2% (2 out of 9). Overall, the combined location rates of OXRSA (50.7%; 37 out of 73) and OXSSA (49.3%; 36 out of 73) were evenly distributed.

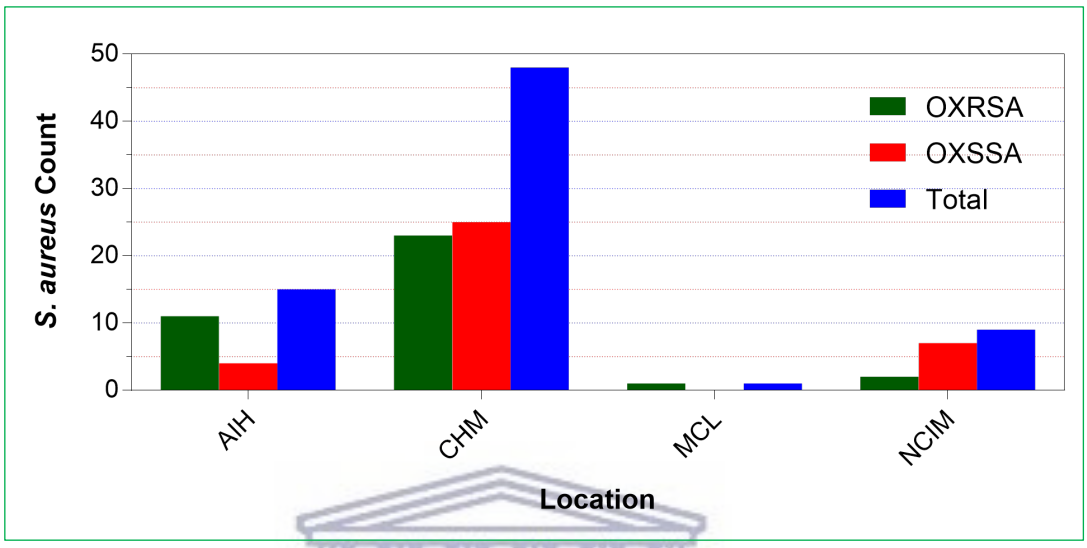


Figure 3.19: *S. aureus* resistance and sensitivity to oxacillin in samples according to location

The statistics show that the difference between location with respect to and OXSSA is insignificant (Chi-Square=0.068). In the present investigation, the overall OXRSA (MRSA) rate of 50.7% (n=73) in isolates from all the locations agrees with the 51% MRSA resistance to oxacillin (n=170) reported by Ahmed et al. (2010). Clearly, one should detect MRSA before hospitalization or the transfer (referral) to other health care centers (Romaniszyn et al., 2014).

3.1.8.9.2 *S. aureus* Vancomycin-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.70 and Figure 3.20 show the comparison of *S. aureus* resistance and sensitivity to vancomycin in samples according to location, i.e., a private laboratory and three hospitals. The rate of VSSA exceed 90% in samples collected from all locations, i.e., AIH (93.3%;14 out of 15), CHM (95.8%; 46 out of 48), MCL (100%; 1 out of 1) and MCIM (100%; 9 out of 9). VRSA rates of 6.7% (1 out of 15) and 4.2% (2 out of 48) were observed for AIH and CHM,

respectively, but not in the other locations. The statistics show that the difference between location with respect to VRSA and VSSA is insignificant (Chi-Square 0.878). The detection of VRSA may be due to several factors, including indiscriminate prescription and use of antibiotics, lack of adherence to appropriate sterilization policies and procedures (Anupurba et al., 2003). VRSA may be brought about by inappropriate handling of patients hospitalized after accidents (Buzaid et al., 2011).

Table 3.70: Comparison of *S. aureus* resistance and sensitivity to vancomycin in samples according to location

Location		Vancomycin		
		VRSA	VSSA	Total
AIH	n	1	14	15
	%	6.7%	93.3%	100%
CHM	n	2	46	48
	%	4.2%	95.8%	100%
MCL	n	0	1	1
	%	0.0%	100%	100%
NCIM	n	0	9	9
	%	0.0%	100%	100%
Total	n	3	70	73
	%	4.1%	95.9%	100%

VRSA=vancomycin-resistant *S. aureus*; VSSA=vancomycin-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	0.678	3	0.878
Likelihood Ratio	1.050	3	0.789
No. of Valid Cases	73		

Our observed overall VRSA rate of 4.1% (3 out of 73) for all locations did not agree with the 0% MRSA resistance to vancomycin reported by Pandya et al. (2014). In this study, it was found that the highest sensitivity to vancomycin was (VSSA) amounted to 95.9% from four locations, which is supported by Leibler et al. (2017) and Hassanzadeh et al. (2013).

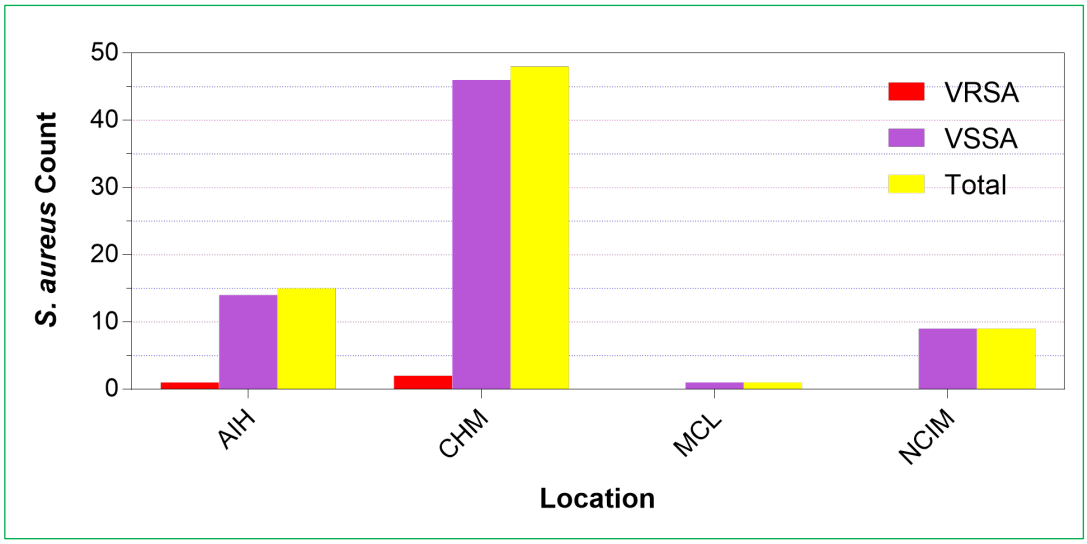


Figure 3.20: *S. aureus* resistance and sensitivity to vancomycin in samples according to location

3.1.8.9.3 *S. aureus* Tetracycline-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.71 and Figure 3.21 show the comparison of *S. aureus* resistance and sensitivity to tetracycline in samples according to location, i.e., a private laboratory and three hospitals. TRSA was identified in all samples collected from all locations, i.e., AIH (40%; 6 out of 15), CHM (56.3%; 27 out of 48), MCL (100%; 1 out of 1) and NCIM (22.2%; 2 out of 9). Samples collected from NCIM yielded the highest TSSA rate (77.8%; 7 out of 9), followed by AIH (60.0%; 9 out of 15), CHM (43.8%; 21 out of 48). No TSSA was observed in samples from MCL. Overall, the rates for TRSA and TSSA were equally distributed, i.e., 49.3% (36 out of 73) and 50.7% (37 out of 73). The statistics show that the difference between location with respect to TRSA and TSSA is insignificant (Chi-Square 0.164). Our observed TRSA rates are lower than that reported by Tiwari et al. (2008) and Orrett & Land (2006), i.e., 82% TRSA of a total of 150 samples tested and 78.7%, respectively. Also, in this study, our overall TRSA rate of 49.3% does not agree with a study by Roberts et al. (2018) in which a total of 13 samples tested no antibiotic resistance to tetracycline. However, our TSSA rate observed for samples derived from NCIM (77.8%; 7 out of 9) agrees with a result by Jamali et al. (Jamali et al., 2017) in which TSSA was reported as 76.7% (n=43) in bovine samples.

Table 3.71: Comparison of *S. aureus* resistance and sensitivity to tetracycline in samples according to location

Location		Tetracycline		
		TRSA	TSSA	Total
AIH	n	6	9	15
	%	40.0%	60.0%	100%
CHM	n	27	21	48
	%	56.3%	43.8%	100%
MCL	n	1	0	1
	%	100%	0.0%	100%
NCIM	n	2	7	9
	%	22.2%	77.8%	100%
Total	n	36	37	73
	%	49.3%	50.7%	100%

TRSA=tetracycline-resistant *S. aureus*; TSSA=tetracycline-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.115	3	0.164
Likelihood Ratio	5.671	3	0.129
No. of Valid Cases	73		

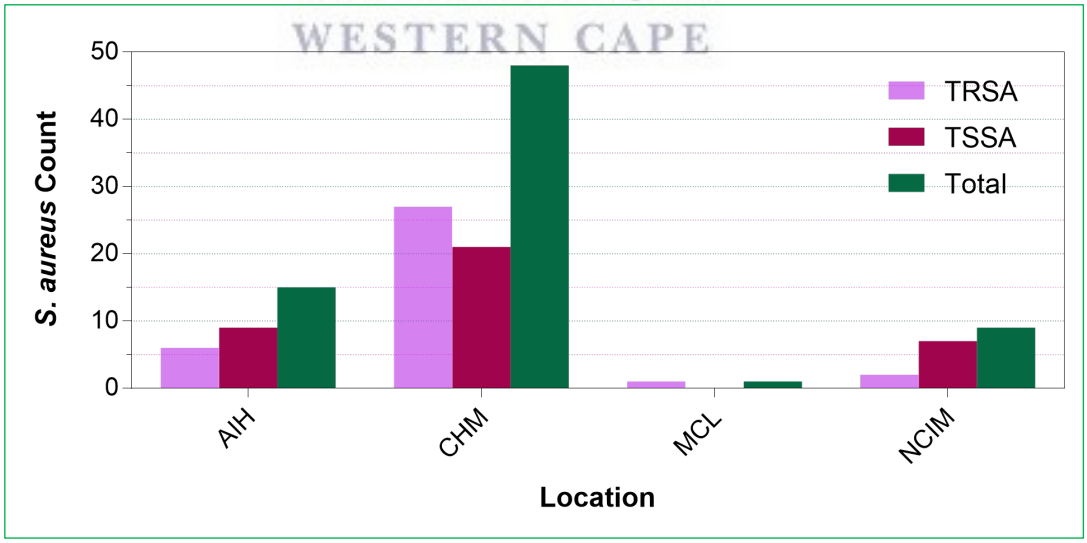


Figure 3.21: *S. aureus* resistance and sensitivity to tetracycline in samples according to location

3.1.8.9.4 *S. aureus* Erythromycin-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.72 and Figure 3.22 show the comparison of *S. aureus* resistance and sensitivity to erythromycin in samples according to location, i.e., a private laboratory and three hospitals.

Table 3.72: Comparison of *S. aureus* resistance and sensitivity to erythromycin in samples according to location

Location		Erythromycin			
		EISSA	ERSA	ESSA	Total
AIH	n	0	11	4	15
	%	0.0%	73.3%	26.7%	100%
CHM	n	1	34	13	48
	%	2.1%	70.8%	27.1%	100%
MCL	n	0	1	0	1
	%	0.0%	100%	0.0%	100%
NCIM	n	0	8	1	9
	%	0.0%	88.9%	11.1%	100%
Total	n	1	54	18	73
	%	1.4%	74.0%	24.7%	100%

EISSA=erythromycin-intermediate-sensitive *S. aureus*; ERSA=erythromycin-resistant *S. aureus*; ESSA=erythromycin-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.003	6	0.919
Likelihood Ratio	2.713	6	0.844
No. of Valid Cases	73		

EISSA was detected in only one sample from CHM (2.1%; 1 out of 48) while the ERSA rate was relatively high in samples collected from all locations, i.e., AIH (73.3%; 11 out of 15), CHM (70.8%; 34 out of 48), MCL (100%; 1 out of 1) and NCIM (88.9%; 8 out of 9) compared to correspondingly lower ESSA levels, i.e., AIH (26.7%; 4 out of 15), CHM (27.1%; 13 out of 48) and NCIM (11.1%; 1 out of 9). No ESSA was observed in samples from MCL. The statistics show that the difference between location with respect to ERSA, ESSA and EISSA is insignificant (Chi-Square 0.919). Our overall result of 74% ERSA (54 out of 73 samples)

for all locations, agrees with a study by Mohanasoundaram et al. (2008) in which ERSA was 85% of the total 150 samples tested. It also agrees with the studies by Orrett & Land (2006) and Leibler et al. (2017), which found 86.7% and 79.2% ERSA, respectively.

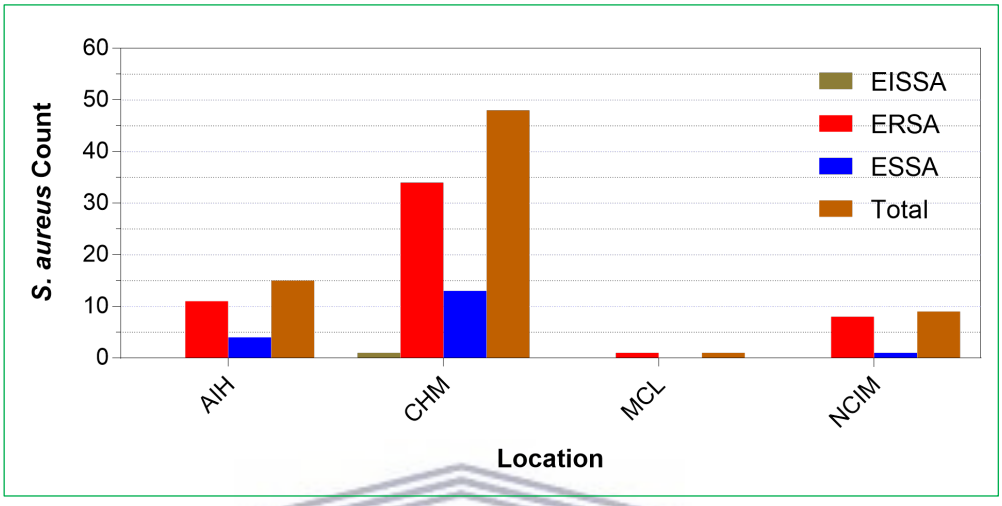


Figure 3.22 *S. aureus* resistance and sensitivity to erythromycin in samples according to location

3.1.8.9.5 *S. aureus* Gentamicin-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.73 and Figure 3.23 show the comparison of *S. aureus* resistance and sensitivity to gentamicin in samples according to location, i.e., a private laboratory and three hospitals. GISSA rates of 4.2% (2 out of 48) and 11.1% (1 out of 9) were observed for samples collected from CHM and NCIM, respectively. Thus, the overall the GISSA rate was low for samples collected from all locations (4.1%; 3 out of 73). One sample each collected from locations AIH and MCL yielded GRSA at rates of 6.7% (1 out of 15) and 100% (1 out of 1), respectively, whereas samples collected from CHM showed GRSA at a rate of 18.8% (9 out of 48). No GRSA was detected in samples from NCIM. The GSSA rates were high in samples collected from AIH (93.3%; 14 out of 15), CHM (77.1%; 37 out of 48), NCIM (88.9%; 8 out of 9), except in samples from MCL for which no GSSA was detected. The statistics show that the difference between location with respect to GRSA, GSSA and GISSA is insignificant (Chi-Square=0.131). Our GRSA results are substantially lower compared to reported values of 88%

of a total of 150 samples (Mohanasoundaram & Lalitha, 2008), while Rajaduraipandi et al. (2006) observed a GRSA rate of 63.2% in their study.

Table 3.73: Comparison of *S. aureus* resistance and sensitivity to gentamicin in samples according to location

Location		Gentamicin			
		GISSA	GRSA	GSSA	Total
AIH	n	0	1	14	15
	%	0.0%	6.7%	93.3%	100%
CHM	n	2	9	37	48
	%	4.2%	18.8%	77.1%	100%
MCL	n	0	1	0	1
	%	0.0%	100%	0.0%	100%
NCIM	n	1	0	8	9
	%	11.1%	0.0%	88.9%	100%
Total	n	3	11	59	73
	%	4.1%	15.1%	80.8%	100%

GISSA=gentamicin-intermediate-sensitive *S. aureus*; GRSA=gentamicin-resistant *S. aureus*; GSSA=gentamicin-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

Chi-Square Tests	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	10.263[a]	6	0.114	0.131
Likelihood Ratio	10.181	6	0.117	0.093
Fisher's Exact Test	9.267			0.148
No. of Valid Cases	73			

[a]8 cells (66.7%) have expected count less than 5. The minimum expected count is 0.04.

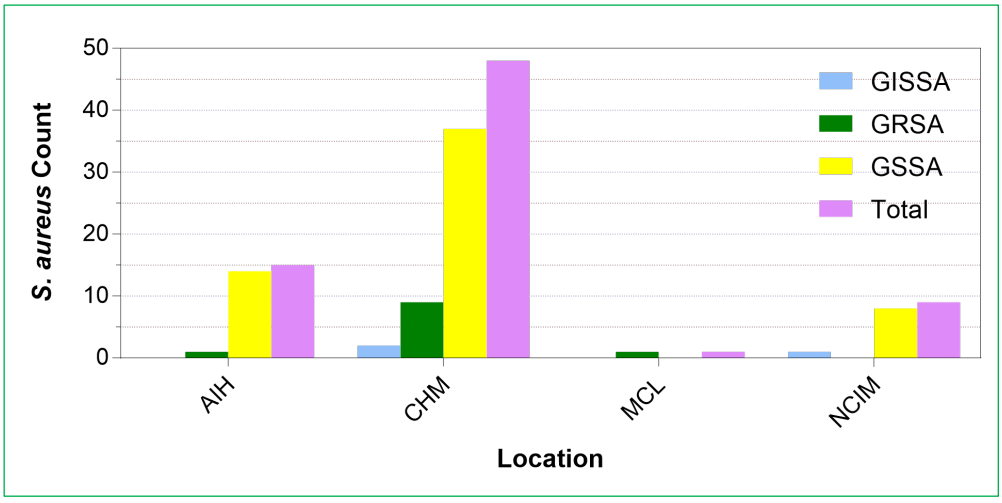


Figure 3.23: *S. aureus* resistance and sensitivity to gentamicin in samples according to location

3.1.8.9.6 *S. aureus* Kanamycin-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.74 and Figure 3.24 show the comparison of *S. aureus* resistance and sensitivity to kanamycin in samples according to location, i.e., a private laboratory and three hospitals. The statistics show that the difference between location with respect to KRSA and KSSA is insignificant (Chi-Square=0.127). High rates of KRSA was observed in samples collected from CHM (58.3%; 28 out of 48) and NCIM (55.6%; 5 out of 9), and a lower rate in AIH (26.7%; 4 out of 15), whereas MCL samples yielded no KRSA.

Similarly, KSSA was comparatively high in samples derived from all locations, i.e., AIH (73.3%; 11 out of 15), CHM (41.7%; 20 out of 48), MCL (100%; 1 out of 1) and NCIM (44.4%; 4 out of 9). Overall, the KRSA (50.7%; 37 out of 73) and KSSA (49.3%; 36 out of 73) rates were evenly distributed in the sample frame. Our results are consistent with a study by Edslev et al. (2018) which found strains of *S. aureus* that were susceptible to kanamycin.

3.1.8.9.7 *S. aureus* Streptomycin-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.75 and Figure 3.25 show the comparison of *S. aureus* resistance and sensitivity to streptomycin in samples according to location, i.e., a private laboratory and three hospitals. SRSA was detected in samples collected from all locations, i.e., AIH (40.0%; 6 out of 16), CHM (6.3%; 3 out of 48), MCL (100%; 1 out of 1) and NCIM (33.3%; 3 out of 9). High rates of SSSA were observed for samples from all locations, i.e., AIH (60.0%; 9 out of 15), CHM (93.8%; 45 out of 48), and NCIM (66.7%; 6 out of 9), but SSSA was not detectable in samples from MCL.

Overall, the SRSA rate (17.8%; 13 out of 73) was low compared to the SSSA (82.2%; 60 out of 73) in the sampling frame. The statistics show that the difference between location with respect to SRSA and SSSA is significant (Chi-Square=0.001).

Table 3.74: Comparison of *S. aureus* resistance and sensitivity to kanamycin in samples according to location

Location		Kanamycin		
		KRSA	KSSA	Total
AIH	n	4	11	15
	%	26.7%	73.3%	100%
CHM	n	28	20	48
	%	58.3%	41.7%	100%
MCL	n	0	1	1
	%	0.0%	100%	100%
NCIM	n	5	4	9
	%	55.6%	44.4%	100%
Total	n	37	36	73
	%	50.7%	49.3%	100%

KRSA=kanamycin-resistant *S. aureus*; KSSA=kanamycin-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.698	3	0.127
Likelihood Ratio	6.220	3	0.101
No. of Valid Cases	73		

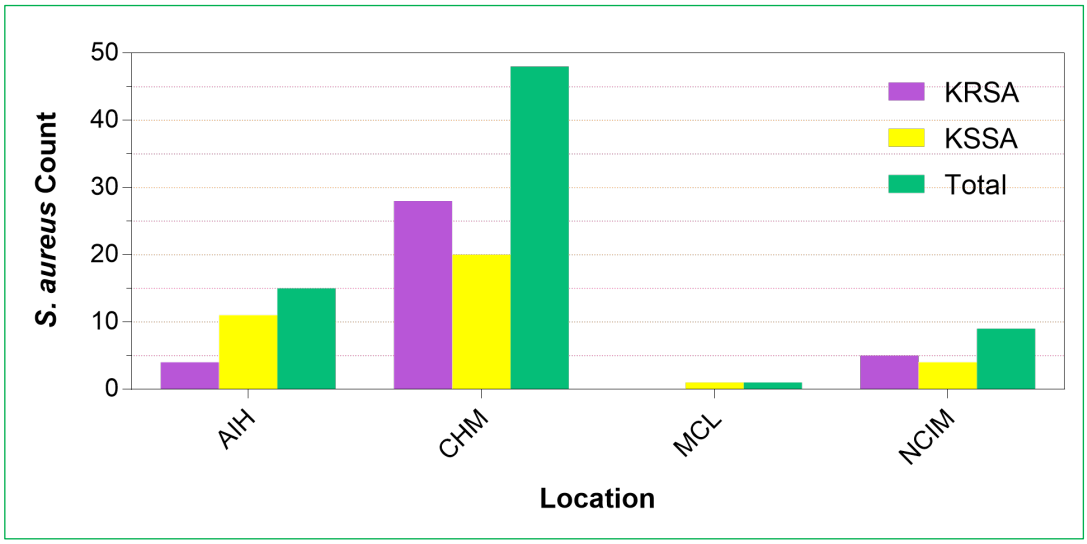


Figure 3.24: *S. aureus* resistance and sensitivity to kanamycin in samples according to location

Table 3.75: Comparison of *S. aureus* resistance and sensitivity to streptomycin in samples according to location

Location		Streptomycin		
		SRSA	SSSA	Total
AIH	n	6	9	15
	%	40.0%	60.0%	100%
CHM	n	3	45	48
	%	6.3%	93.8%	100%
MCL	n	1	0	1
	%	100%	0.0%	100%
NCIM	n	3	6	9
	%	33.3%	66.7%	100%
Total	n	13	60	73
	%	17.8%	82.2%	100%

SRSA=streptomycin-resistant *S. aureus*; SSSA=streptomycin-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.525[a]	3	0.001
Likelihood Ratio	14.305	3	0.003
No. of Valid Cases	73		

[a]4 cells (50.0%) have expected count less than 5. The minimum expected count is 0.18.

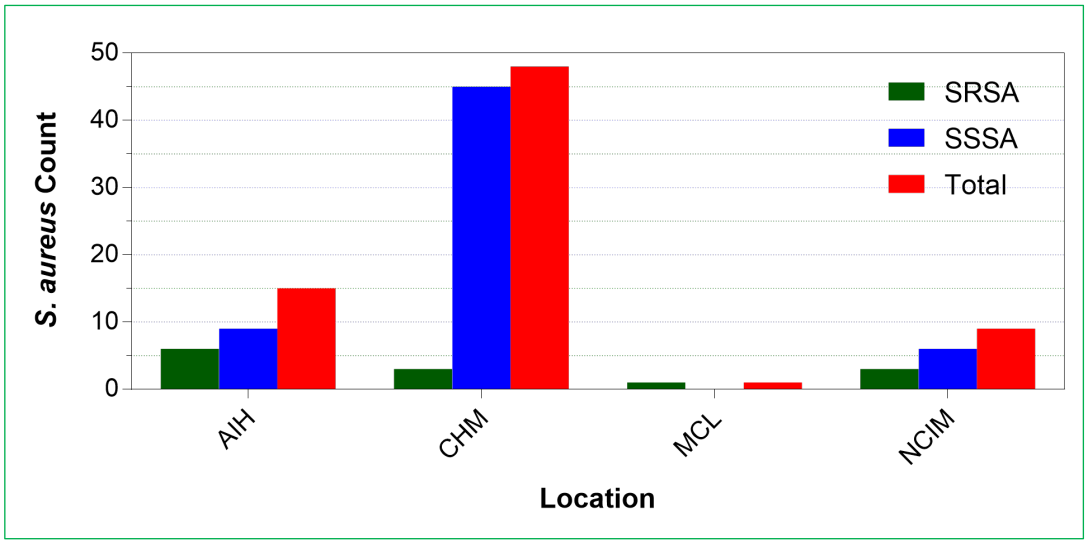


Figure 3.25: *S. aureus* resistance and sensitivity to streptomycin in samples according to location

3.1.8.9.8 *S. aureus* Amikacin-Resistant and-Sensitive Profiles in Samples According to Location

Table 3.76 and Figure 3.26 show the comparison of *S. aureus* resistance and sensitivity to amikacin in samples according to location, i.e., a private laboratory and three hospitals. AISSA was detected in one sample collected from AIH (6.7%; 1 out of 15), but not in samples collected from any of the other locations. ARSA was also not observed in samples collected from MCL, but samples from other locations yielded very low ARSA rates, i.e., AIH (13.3%; 2 out of 15), CHM (8.3%; 4 out of 48) and NCIM (11.1%; 1 out of 9). By contrast, the ASSA was very high in samples sourced from all locations, i.e., AIH (80.0%; 12 out of 15), CHM (91.7%; 44 out of 48), MCL (100%; 1 out of 1) and NCIM (88.9%; 8 out of 9). Overall, the ASSA rate (89.0%; 65 out of 73) exceeded the ARSA rate (9.6%; 7 out of 73). The statistics show that the difference between location with respect to ARSA and ASSA is insignificant (Chi-Square=0.613).

3.1.8.9.9 PCR Profiles of *S. aureus*-Resistance and-Sensitivity in Samples According to Location

Table 3.77 and Figure 3.27 show the PCR profiles of *S. aureus*-resistance and-sensitivity in samples according to location, i.e., a private laboratory and three hospitals. Samples collected from most locations showed PCR+ results, i.e., AIH (100%; 10 out of 10), CHM (95.7%; 22 out of 23) and NCIM (100%; 2 out of 2), but samples collected from MCL all showed PCR- results (100%; 1 out of 1). Low PCR- results were also detected in samples sourced from CHM (4.3%; 1 out of 23). Overall, the PCR+ count (94.4%; 34 out of 36) exceeded the PCR- count (5.6%; 2 out of 36). The results of the present study showed that traditional methods of disk diffusion and MIC strips, using oxacillin, was efficient in the detection of MRSA because the error of PCR- which accounted for 5.6% agreed with the study by Mohanasoundaram et al. (2008) in which one strain of MSSA showed a negative PCR due to borderline resistance linked to upregulation of beta-lactamases1 (Foster, 2018). Statistically, the difference between location with respect to PCR- and PCR+ is significant (Chi-Square=0.000).

Table 3.76: Comparison of *S. aureus* resistance and sensitivity to amikacin in samples according to location

Location		Amikacin			
		AISSA	ARSA	ASSA	Total
AIH	n	1	2	12	15
	%	6.7%	13.3%	80.0%	100%
CHM	n	0	4	44	48
	%	0.0%	8.3%	91.7%	100%
MCL	n	0	0	1	1
	%	0.0%	0.0%	100%	100%
NCIM	n	0	1	8	9
	%	0.0%	11.1%	88.9%	100%
Total	n	1	7	65	73
	%	1.4%	9.6%	89.0%	100%

AISSA=amikacin-intermediate-sensitive *S. aureus*; ARSA=amikacin-resistant *S. aureus*; ASSA=amikacin-sensitive *S. aureus*; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.471	6	0.613
Likelihood Ratio	3.848	6	0.697
No. of Valid Cases	73		

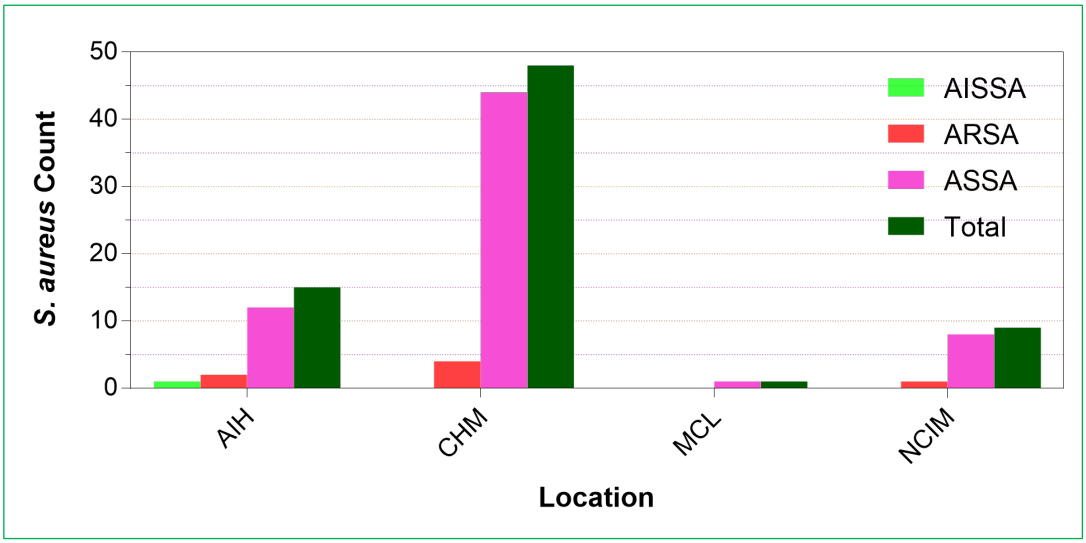


Figure 3.26: *S. aureus* resistance and sensitivity to amikacin in samples according to location

Table 3.77: PCR profiles of *S. aureus*-resistance and-sensitivity in samples according to location

Location		Polymerase Chain Reaction (PCR)		
		PCR-	PCR+	Total
AIH	n	0	10	10
	%	0.0%	100%	100%
CHM	n	1	22	23
	%	4.3%	95.7%	100%
MCL	n	1	0	1
	%	100%	0.0%	100%
NCIM	n	0	2	2
	%	0.0%	100%	100%
Total	n	2	34	36
	%	5.6%	94.4%	100%

PCR-=PCR negative/ no *mecA1* and *mecA2* genes; PCR+=PCR positive/*mecA1 and mecA2* genes; AIH=Assafwa International Hospital; CHM=Central Hospital Misurata; MCL=Misurata Central Laboratory; NCIM=National Cancer Institute Misurata

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.770	3	0.000
Likelihood Ratio	7.221	3	0.065
No. of Valid Cases	36		

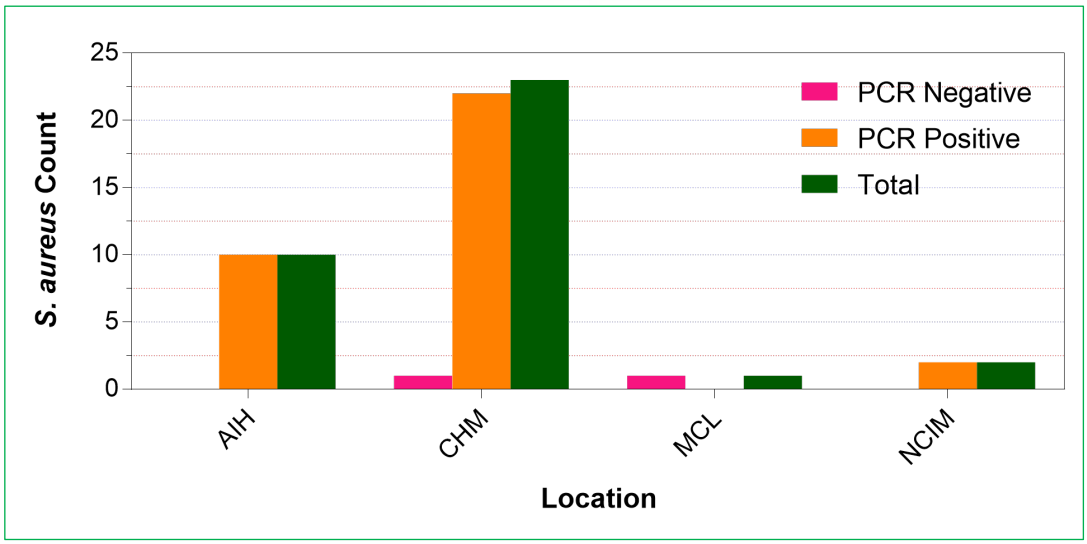


Figure 3.27: PCR profiles of *S. aureus*-resistance and-sensitivity in samples according to location

3.2 Analysis of Poultry Samples

3.2.1 Introduction

Considering that *Staphylococcus aureus* is an important food-borne and meat-borne pathogen (Silbergeld et al., 2008), this study sought to investigate the potential health hazard of chicken meat products as well as that of the wider prevalent environment. Several studies have provided evidence of the transfer of resistance genes from animals to the humans, whether by contact, water linked to animals or by food (Teuber, 2001), or the possibility of human pollution of poultry and chicken carcasses in slaughterhouses (Persoons et al., 2009). In addition, identical strains of vancomycin-resistant enterococci (VRE) were found both in humans and animals (Dahms et al., 2014).

An identity between RAPD (Random Amplification of Polymorphic DNA) of 6 isolates from animals when compared with isolates of similar types from humans was discovered. A close relation between genomes of the six animal MRSA were shown by the antibiotic types of the six animal isolates (Lee, 2003). Moreover, another study by Álvarez-Fernández et al. (2012) found *Salmonella* in 55% of the samples in 1993, 12.4% in 2006 from chicken wings, necks, carcasses and legs. Additionally, a study by Owuna et al. (2015) found 29 (72.5%) of *S. aureus* from 40 samples of chicken meat. The objectives of this section of our study were to determine the prevalence in and translation of *S. aureus* to humans.

3.2.2 Collection and of Samples

We collected 361 swabs from the different locations of which 72 showed no bacterial growth, while 289 showed bacterial growth. Regarding the live chickens slaughtered by myself in the Misurata City Laboratory, we took swabs after slaughter from the outer neck, deep inside the neck, under the wings, under the thighs and from the inside of the external os, all under sterile conditions. The tests used were blood agar culture (BAC), mannitol salt agar (MSA) and Muller-Hinton agar (MHA), the biochemical tests for coagulase, catalase and Gram-stain.

3.2.3 Bacterial Growth in Poultry Samples Collected from Various Locations

Table 3.78 and Figure 3.28 show the distribution of bacterial growth in the poultry samples collected from various locations.

Table 3.78: Bacterial growth in all poultry samples

Sample	Bacterial Growth Tests		
	Growth	No Growth	Total
n	289	72	361
%	80.06%	19.94%	100%

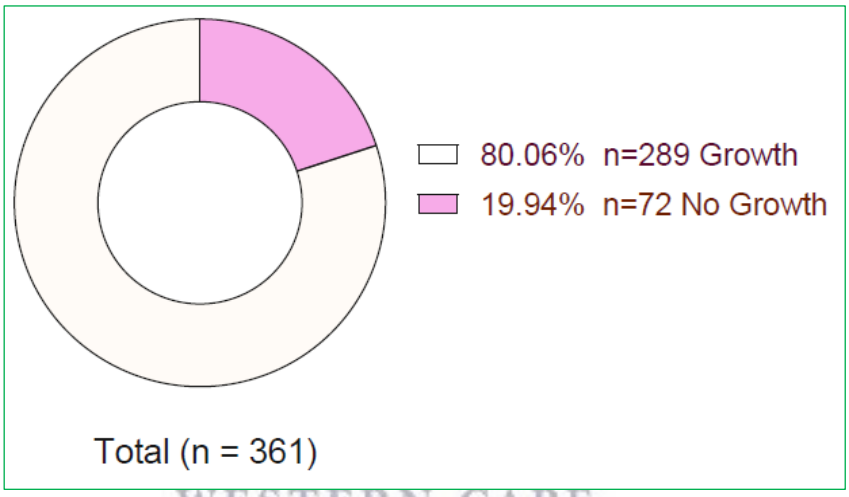


Figure 3.28: Bacterial growth in all poultry samples

Of the 361 swabs taken from five locations, 72 (19.94%) showed no bacterial growth, while 289 (80.06%) showed bacterial growth.

3.2.4 Bacterial Growth in Poultry Samples According to Location

Table 3.79 and Figure 3.29 show the number of poultry samples collected and tested according to location. The highest number of specimens was collected from the location 5 (36%; n=104), followed by the locations 3 (27%; n=78) and 4 (17%; n=50). The lowest number collected was from the location 2 (8%; n=23), with the number from location 1 (12%; n=34) being the second lowest.

Table 3.79: Bacterial growth in poultry samples according to location

Location	Frequency (n)	Percent
1. Abdurrahman Abahy	34	12%
2. Alfetory	23	8%
3. Baser	78	27%
4. Isolate	50	17%
5. Alkherobp Market	104	36%
Total	**289**	100%

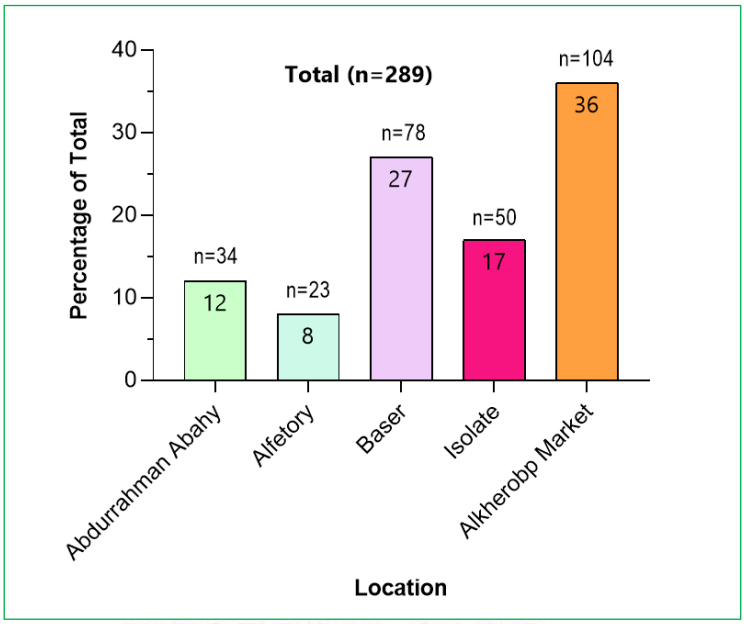


Figure 3.29: Bacterial growth in poultry samples according to location

3.2.5 Bacterial Growth in Different Parts of Poultry Samples

Table 3.80 and Figure 3.30 show the bacterial growth in different parts of poultry samples after slaughter. Bacterial growth was the highest in FURT (21%; 61 out of 289), followed by FULT (19%; 55 out of 289), FURW (19%; 54 out of 289), FULW (17%; 49 out of 289), IEO (16%; 45 out of 289), INAS (7%; 20 out of 289) and FNAS (2%; 5 out of 289).

Table 3.80: Bacterial growth in different parts of poultry samples after slaughter

Part of chicken from which swab sample was taken	Abbreviation Used	Frequency (n)	Percent
From neck after slaughter	FNAS	5	2%
From under left wing	FULW	49	17%
From under right wing	FURW	54	19%
From under the left thigh	FULT	55	19%
From under the right thigh	FURT	61	21%
Inside external os of the chicken	IEO	45	16%
Inside neck after slaughter	INAS	20	7%
Total		**289**	100%

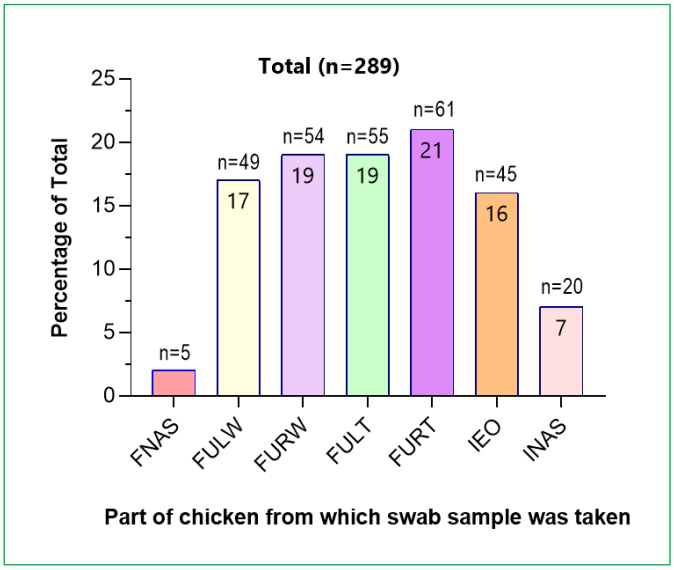


Figure 3.30: Bacterial growth in different parts of poultry samples after slaughter

3.2.6 Bacterial Growth in Blood Agar Cultures of Poultry Samples

Table 3.81 and Figure 3.31 show bacterial growth in blood agar cultures of poultry samples. BHB (64%; 185 out of 289) represented the highest fraction of bacterial growth, followed by BNHB (27%; 79 out of 289) and *Proteus* (9%; 25 out of 289).

Table 3.81: Bacterial growth in blood agar cultures of poultry samples

Bacterial Growth in Blood Agar Culture	Abbreviation Used	Frequency (n)	Percent
Proteus	Proteus	25	9%
Bacteria Haemolytic Blood	BHB	185	64%
Bacteria Non-Haemolytic Blood	BNHB	79	27%
Total		**289**	100%

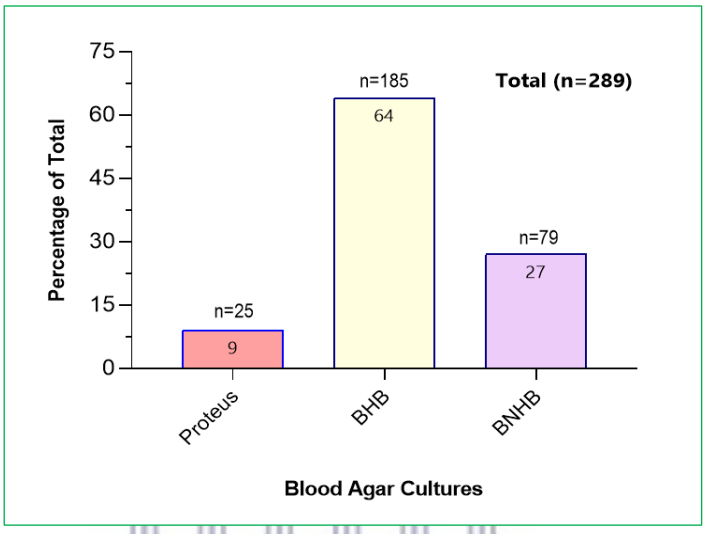


Figure 3.31: Bacterial growth in blood agar cultures of poultry samples

3.2.7 Bacterial Growth in Mannitol Salt Agar Cultures of Poultry Samples

Table 3.82 and Figure 3.32 show the bacterial growth in mannitol salt agar (MSA) cultures of poultry samples.

Table 3.82: Bacterial growth in mannitol salt agar cultures of poultry samples

Bacterial Growth in Mannitol Salt Agar	Abbreviation Used	Frequency (n)	Percent
Growth of bacteria	GB	7	2%
Bacteria fermentation mannitol salt agar	BFMSA	226	78%
Bacteria non-fermentation mannitol salt agar	BNFMSA	56	20%
Total		**289**	100%

BFMSA (78%; 226 out of 289) was the highest portion of bacterial growth in MSA, followed by BNFMSA (20%; 56 out of 289) and GB (2%; 7 out of 289).

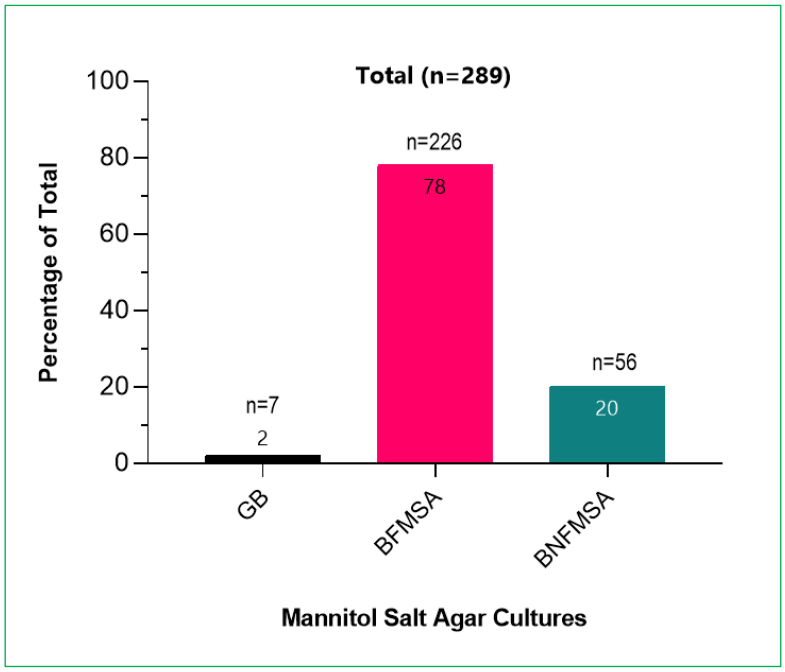


Figure 3.32: Bacterial growth in mannitol salt agar cultures of poultry samples

3.2.8 Gram-stain, Catalase and Coagulase Test Results for Cultures of Poultry Samples

Table 3.83 and Figure 3.33 show the Gram-stain, catalase and coagulase test results for cultures of poultry samples.

Table 3.83: Gram-stain, catalase and coagulase test results for cultures of poultry samples

Test		Negative	Positive	No Test	Total
Gram-stain	n	87	202	0	**289**
	%	30%	70%	0%	100%
Catalase	n	31	224	34	**289**
	%	11%	77%	12%	100%
Coagulase	n	152	52	85	**289**
	%	53%	18%	29%	100%
Bacteria Gram-Stain-Negative=GBN-(*Enterobacteriaceae*+*Pseudomonas*); Bacteria Gram-Stain-Positive=GPB+; Bacteria Catalase-Negative=BCN-; Bacteria Catalase-Positive=BCP+; Bacteria Coagulase-Negative=BCGN; Bacteria Coagulase-Positive=BCGP					

Poultry samples tested showed more Gram-stain-positive (70%; 202 out of 289) than Gram-stain-negative (30%; 87 out of 289). The number of catalase-positive samples (77%; 224 out

of 289) exceeded that of catalase-negative (11%; 31 out of 289), while no test results were also observed (12%; 34 out of 289). By contrast, the number of coagulase-negative (53%; 152 out of 289) exceeded that of the coagulase-positive (18%; 52 out of 289), while there were also no test results (29%; 85 out of 289).

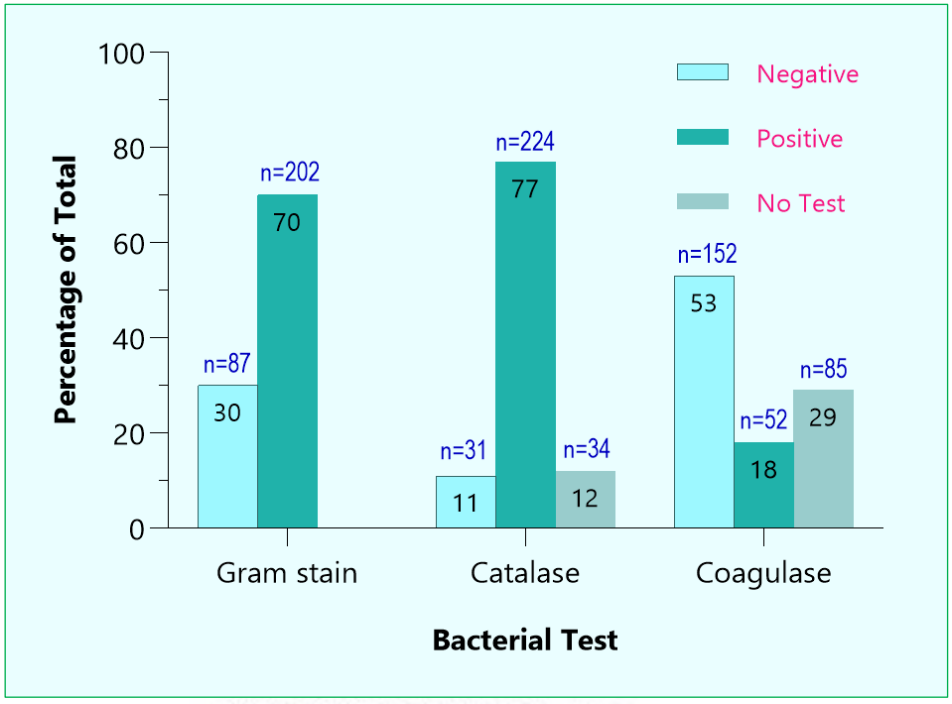


Figure 3.33: Gram-stain, catalase and coagulase test results for cultures of poultry samples

3.2.9 Bacterial Diagnostic Test Results for Cultures of Poultry Samples

Table 3.84 and Figure 3.34 show the bacterial diagnostic test results for cultures of poultry samples.

Table 3.84: Bacterial diagnostic test results for cultures of poultry samples

Bacterial Diagnostic Test	Abbreviation	n	%
Bacteria Gram-Negative (*Enterobacteriaceae*+*Pseudomonas*)	BGN	87	30.1%
Staphylococcus aureus	*S. aureus*	52	18.0%
Staphylococcus Coagulase Negative	SCN	144	49.8%
Streptococcus	*Streptococcus*	6	2.1%
Total		**289**	100%

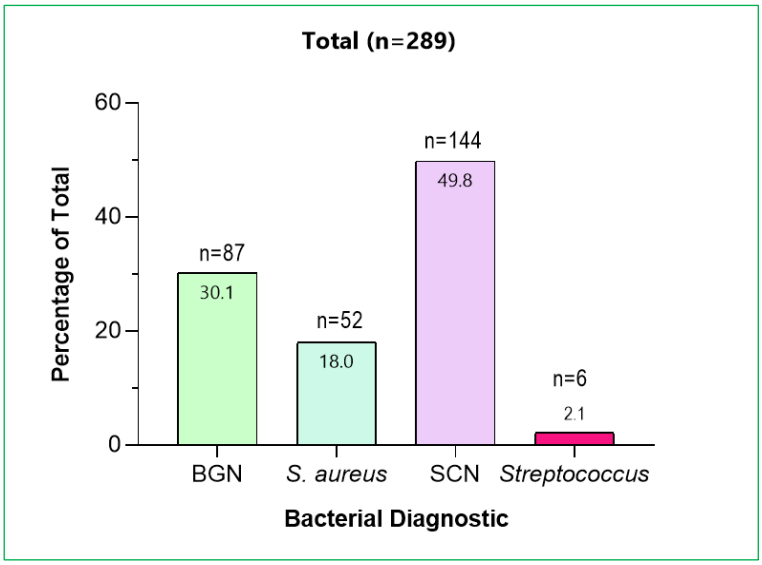


Figure 3.34: Bacterial diagnostic test results for cultures of poultry samples

Bacterial diagnostic test results for cultures of poultry samples revealed the following frequencies in descending order: SCN (49.8%; 144 out of 289), BGN (30.1%; 87 out of 289), *S. aureus* (18.0%; 52 out of 289) and *Streptocoocus* (2.1%; 6 out of 289).

3.2.10 Bacterial Growth in Mueller-Hinton Agar Cultures of Poultry Samples

Table 3.85 and Figure 3.35 show the bacterial growth in Mueller-Hinton agar cultures of poultry samples.

Table 3.85: Bacterial growth in Mueller-Hinton agar cultures of poultry samples

Sample	Mueller-Hinton Agar Cultures		
	Positive	No Test	Total
n	48	4	52
%	92.3%	7.7%	100%

In the Mueller-Hinton agar test, 92.3% (48 out of 52) of the poultry samples were positive for bacterial growth, whereas 7.7% (4 out of 52) represented no test results.

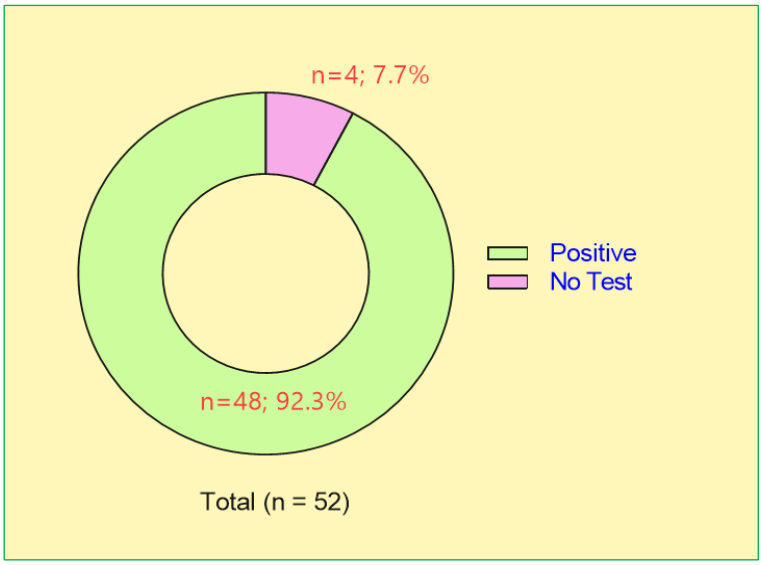


Figure 3.35: Bacterial growth in Mueller-Hinton agar cultures of poultry samples

3.2.11 Comparison of *S. aureus* Antibiotic Resistance and Sensitivity Profiles in Poultry Samples

Table 3.86 shows the *S. aureus* antibiotic resistance and sensitivity profiles in poultry samples collected from the 5 locations.

Table 3.86: *S. aureus* antibiotic resistance and sensitivity profiles in poultry samples

Antibiotic	ARSA		ASSA		AISA		Total	
	n	%	n	%	n	%	n	%
Oxacillin	0	0%	48	100%	0	0%	48	100%
Vancomycin	2	4%	46	96%	0	0%	48	100%
Tetracycline	26	54.2%	17	35.4%	5	10.4%	48	100%
Erythromycin	34	71%	13	27%	1	2%	48	100%
Gentamicin	3	6%	45	94%	0	0%	48	100%
Kanamycin	13	27%	35	73%	0	0%	48	100%
Streptomycin	5	10%	43	90%	0	0%	48	100%
Amikacin	0	0%	48	100%	0	0%	48	100%

Antibiotic-Resistant *S. aureus*=ARSA; Antibiotic-Sensitive *S. aureus*=ASSA; Antibiotic-Intermediate-Sensitive *S. aureus*=AISSA

Of the 48 samples tested for *S. aureus* antibiotic sensitivity, all were sensitive to oxacillin (100%; 48 out of 48), whereas 96% were sensitive to vancomycin (46 out of 48), 35.4% were

sensitive to tetracycline (17 out of 48) and 10.4% showed intermediate sensitivity to tetracycline (5 out of 48), 94% were sensitive to gentamicin (45 out of 48), 73% to kanamycin (35 out of 48), 90% to streptomycin (43 out of 48) and 100% to amikacin (48 out of 48). *S. aureus* antibiotic-resistance were not observed for oxacillin and amikacin, but for vancomycin (4%; 2 out of 48), tetracycline (54.5%; 26 out of 48), erythromycin (71%; 34 out of 48), gentamicin (6%; 3 out of 48), kanamycin (27%; 13 out of 48) and streptomycin (10%; 5 out of 48).

We did not find *S. aureus* oxacillin-resistant strains in any of the poultry samples. This does not agree with a study by Gundogan et al. (2005), in which it was found that the rates of *S. aureus* resistance to methicillin and erythromycin were of the order of 67.5% and 7.5%, respectively. It is generally held that the presence of antimicrobes in live chickens before slaughtering are much less than after slaughtering and selling (Mulders et al., 2010). Moreover, it is due to the prevalence of the microorganism's resistance, including resistance to clinically important antibiotics, such as erythromycin and oxacillin (Waters et al., 2011).

Our results agree with a study by Pesavento et al. (2007), in which 42 isolates of *S. aureus* from 176 samples of raw meat (poultry, beef and pork) exhibited no resistance to methicillin. Darwish et al. (2018) documented 78.57% MRSA demonstrated by testing coagulase-positive samples from chicken breast meat, wings, giblets and liver, which is consonant with an earlier study (Gundogan et al., 2005). Moreover, a study by Abdalrahman et al. (2015) who observed a rate of 0.9% MRSA of the total 167 samples from chicken and turkey meat, has recently been bolstered by similar findings (Ge et al., 2017).

Furthemore, in a study by Sallam et al. (2015) it was found that resistance to penicillin, ampicillin and cloxacillin had counts of 93.4%, 88.9% and 83.3%, respectively, in addition to 38% MRSA of the 200 samples tested for the *mecA* gene by PCR. In addition, our result agrees with a study by Mulders et al. (2010) which compared a total of 405 broilers samples before

slaughter, of which 69% were positive for MRSA, whereas after slaughter 35.0% were positive. MRSA contamination in the different compartments of slaughterhouses increased during the production day, from 8% to 35%. of the 119 MRSA isolates, predominantly livestock-associated MRSA ST398 were found. We found resistance to vancomycin was 4.0%, which agrees with a study by Gundogan et al. (2005) which found that every strain was sensitive to vancomycin. Likewise, our result agrees with the findings of the study by Nworie et al. (2017) in which it was found that *S. aureus* isolates in chicken samples showed no resistance to vancomycin. The study by Nworie et al. (2017) corroborates the work of Gundogan et al. (2005).

Our observed rate of resistance to tetracycline (54.2%; 26 out of 48) agrees with a study by Jamali et al. (2015) in which it was reported that the rate of *S. aureus* resistance to tetracycline was 56.1%. Our results do not agree with the findings of the study by Kim et al. (2018) in which the rate of *S. aureus* resistance to tetracycline was stated as 7 (21.9%) in isolates from chickens. Furthemore, our results agree with the findings of the study by Nworie et al. (2017), viz., the rate of *S. aureus* resistance to tetracycline was 113 (45.7%) in isolates from chickens.

In our study, the resistance of *S. aureus* to gentamicin was 6% (3 out of 48), which agrees with a study by Owuna et al. (2015) who observed that 17.2% of *S. aureus* isolates were resistant gentamicin. By analogy, Kim et al. (2018) recently reported that the rate of *S. aureus* resistance to gentamicin was 1 (3.1%) in isolates from chickens. Also, our results are congruent with the findings of Nworie et al. (2017) in that the rate of *S. aureus* resistance to gentamicin was 13 (5.3%) in isolates from chickens.

Moreover, we found that the resistance of *S. aureus* to kanamycin was 27% (13 out of 48), which is inconsistent with the findings of the study by Jamali et al. (2015) in which *S. aureus* resistance to of kanamycin was 4% in the isolates from dairy milk products. Moreover, our results agree with the findings of Kim et al. (2018) in which the rate of *S. aureus* resistance to

kanamycin was 16 (48.5%) in isolates from chickens. Our finding of *S. aureus* resistance to streptomycin (10%; 5 out of 48) compares well with the 20.7% rate reported for *S. aureus* resistance to streptomycin (Owuna et al., 2015).

3.2.12 Bacterial Growth of Different Parts of Poultry Samples in Blood Agar Cultures

Table 3.87 shows the bacterial growth in blood agar cultures of swabs taken from different parts of poultry samples.

Table 3.87: Bacterial growth in blood agar cultures of swabs taken from different locations of poultry samples

Part of Chicken		Blood Agar Culture			
		Proteus	BHB	BNHB	Total
FNAS	n	0	2	3	5
	%	0.0%	40.0%	60.0%	100%
FULW	n	1	46	2	49
	%	2.0%	93.9%	4.1%	100%
FURW	n	5	49	0	54
	%	9.3%	90.7%	0.0%	100%
FULT	n	1	51	3	55
	%	1.8%	92.7%	5.5%	100%
FURT	n	0	55	6	61
	%	0.0%	90.2%	9.8%	100%
IEO	n	0	14	31	45
	%	0.0%	31.1%	68.9%	100%
INAS	n	0	9	11	20
	%	0.0%	45.0%	55.0%	100%
Total	n	7	226	56	289
	%	2.4%	78.2%	19.4%	100%

Proteus=Proteus; bacteria haemolytic blood=BHB; bacteria non-haemolytic blood=BNHB; From neck after slaughter=FNAS; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	118.392[a]	12	0.000
Likelihood Ratio	110.082	12	0.000
No. of Valid Cases	289		

[a]8 cells (38.1%) have expected count less than 5. The minimum expected count is 0.43.

The highest rate of *Proteus* was in FURW (9.3%; 5 out of 54). Low rates of *Proteus* were observed in FULW (2%; 1 out of 49) and FULT (1.8%; 1 out of 55), whereas *Proteus* was not detected in FNAS, FURT, IEO and INAS. The rates of BHB were very high in FULW (93.9%; 46 out of 49), FURW (90.7%; 49 out of 54), FULT (92.7%; 51 out of 55), FURT (90.2%; 55 out of 61), but moderately in FNAS (40.0%; 2 out of 5), IEO (31.1%; 14 out of 45) and INAS (45.0%; 9 out of 20). Overall, BHB was the highest in samples (78.2%; 226 out of 289), whereas BNHB had a lower score (19.4%; 56 out of 289). The statistics show that the differences between blood cultures of chicken parts is significant (Chi-Square=0.000).

3.2.13 Bacterial Growth of Different Parts of Poultry Samples in Mannitol Salt Agar Cultures

Table 3.88 shows the bacterial growth in mannitol salt agar cultures of swabs taken from different parts of poultry samples. No GB was detected in the FNAS, FURT and INAS parts of the poultry samples tested. However, GB was observed in FULW (2.0%; 1 out of 49), FURW (9.3%; 5 out of 54), FULT (1.8%; 1 out of 55) and IEO (40%; 18 out of 45). BFMSA was detected at high rates in all samples, i.e., FNAS (40%; 2 out of 5), FULW (81.6%; 40 out of 49), FURW (77.8%; 42 out of 54), FULT (80.0%; 44 out of 55), FURT (72.1%; 44 out of 61) and INAS (35.0%; 7 out of 20), but at low rates in IEO (13.3%; 6 out of 45). The BNFMSA rate was high in FNAS (60.0%; 3 out of 5), FURT (27.9%; 17 out of 61), IEO (46.7%; 21 out of 45) and INAS (65.0%; 13 out of 20), but moderate in FULW (16.3%; 8 out of 49), FURW (13.0%; 7 out of 54) and FULT (18.2%; 10 out of 55). Overall, the BFMSA rate (64.0%; 185 out of 289) exceeded the BNFMSA rate (27.3%; 79 out of 289). The statistics show that the differences between bacterial growth rates of specimens of chicken parts in mannitol salt agar is significant (Chi-Square=0.000).

3.2.14 Gram-stain Test Results for Different Parts of Poultry Samples

Table 3.89 shows the Gram-stain test results of swabs taken from different parts of poultry samples.

Table 3.88: Bacterial growth in mannitol salt agar cultures of swabs taken from different parts of poultry samples

Part of Chicken		Mannitol Salt Agar Culture			
		GB	BFMSA	BNFMSA	Total
FNAS	n	0	2	3	5
	%	0.0%	40.0%	60.0%	100%
FULW	n	1	40	8	49
	%	2.0%	81.6%	16.3%	100%
FURW	n	5	42	7	54
	%	9.3%	77.8%	13.0%	100%
FULT	n	1	44	10	55
	%	1.8%	80.0%	18.2%	100%
FURT	n	0	44	17	61
	%	0.0%	72.1%	27.9%	100%
IEO	n	18	6	21	45
	%	40%	13.3%	46.7%	100%
INAS	n	0	7	13	20
	%	0.0%	35.0%	65.0%	100%
Total	n	25	185	79	289
	%	8.7%	64.0%	27.3%	100%

Growth of bacteria (fermentation)=GB; Bacteria fermentation mannitol salt agar=BFMSA; Bacteria non-fermentation mannitol salt agar=BNFMSA; From neck after slaughter=FNAS; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	134.478[a]	12	0.000
Likelihood Ratio	124.880	12	0.000
Linear-by-Linear Association	56.892	1	0.000
No. of Valid Cases	289		

[a]10 cells (47.6.1%) have expected count less than 5. The minimum expected count is 0.12.

Gram-stain-negative bacteria (BGN) were detected at high levels in IEO (80.0%; 36 out of 45), INAS (40.0%; 8 out of 20), FULT (29.1%; 16 out of 55), FULW (20.4%; 10 out of 49) and FNAS (20%; 1 out of 5). However, Gram-stain-positive bacteria (BGP) counts were generally high for all specimens taken from chicken parts, i.e., FNAS (80.0%; 4 out of 5), FULW (79.6%; 39 out of 49), FURW (90.7%; 49 out of 54), FULT (70.9%; 39 out of 55), FURT (82.0%; 50 out of 61), IEO (20.0%; 9 out of 45) and INAS (60.0%; 12 out of 20). Overall, the BGP (69.9%; 202 out of 289) exceeded the BGN (30.1%; 87 out of 289). The statistics show that the

differences between Gram-stain results for various chicken parts is significant (Chi-Square=0.000).

Table 3.89: Gram-stain test results of swabs taken from different parts of poultry samples

Part of Chicken		Gram-stain		
		Negative (BGN)	Positive (BGP)	Total
FNAS	n	1	4	5
	%	20.0%	80.0%	100%
FULW	n	10	39	49
	%	20.4%	79.6%	100%
FURW	n	5	49	54
	%	9.3%	90.7%	100%
FULT	n	16	39	55
	%	29.1%	70.9%	100%
FURT	n	11	50	61
	%	18.0%	82.0%	100%
IEO	n	36	9	45
	%	80.0%	20.0%	100%
INAS	n	8	12	20
	%	40.0%	60.0%	100%
Total	n	87	202	289
	%	30.1%	69.9%	100%

Bacteria Gram-stain-positive=BGP; Bacteria Gram-stain-negative (*Enterobacteriaceae*+*Pseudomonas*)=BGN; From neck after slaughter=FNAS; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	72.009[a]	6	0.000
Likelihood Ratio	69.823	6	0.000
Linear-by-Linear Association	28.869	1	0.000
No. of Valid Cases	289		

[a]2 cells (14.3%) have expected count less than 5. The minimum expected count is 1.51.

3.2.15 Catalase Test Results for Different Parts of Poultry Samples

Table 3.90 shows the catalase test results of swabs taken from different parts of poultry samples. The catalase-negative (BCN) rates were less than 20% in most specimens taken from parts of the chicken samples, i.e., FULW (8.2%; 4 out of 49), FURW (11.1%; 6 out of 54), FULT (14.5%; 8 out of 55), FURT (6.6%; 4 out of 61) and IEO (20.0%; 9 out of 45), whereas FNAS and INAS did not yield any BCN results.

Table 3.90: Catalase test results of swabs taken from different parts of poultry samples

Part of Chicken		Catalase			
		Negative (BCN)	Positive (BCP)	No Test	Total
FNAS	n	0	5	0	5
	%	0.0%	100%	0.0%	100%
FULW	n	4	42	3	49
	%	8.2%	85.7%	6.1%	100%
FURW	n	6	48	0	54
	%	11.1%	88.9%	0.0%	100%
FULT	n	8	41	6	55
	%	14.5%	74.5%	10.9%	100%
FURT	n	4	56	1	61
	%	6.6%	91.8%	1.6%	100%
IEO	n	9	16	20	45
	%	20.0%	35.6%	44.4%	100%
INAS	n	0	16	4	20
	%	0.0%	80.0%	20.0%	100%
Total	n	31	224	34	289
	%	10.7%	77.5%	11.8%	100%

Bacteria catalase-positive=BCP; Bacteria catalase-negative=BCN; From neck after slaughter=FNAS; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	77.512[a]	12	0.000
Likelihood Ratio	75.020	12	0.000
Linear-by-Linear Association	9.284	1	0.002
No. of Valid Cases	289		

[a]6 cells (28.6%) have expected count less than 5. The minimum expected count is 0.54.

The catalase-positive (BCP) rates were very high in all specimens acquired from all chicken parts, i.e., FNAS (100%; 5 out of 5), FULW (85.7%; 42 out of 49), FURW (88.9%; 48 out of 54), FULT (74.5%; 41 out of 55), FURT (91.8%; 56 out of 61), IEO (35.6%; 16 out of 45) and INAS (80.0%; 16 out of 20). However, no test results were observed for FULW (6.1%; 3 out of 49), FULT (10.9%; 6 out of 55), IEO (44.4%; 20 out of 45) and INAS (20.0%; 4 out of 20). Overall, the BCP rate (77.5%; 224 out of 289) exceeded both the no test (11.8%; 34 out of 289) and BCN (10.7%; 31 out of 289) rates. The statistics show that the differences between catalase results for various chicken parts is significant (Chi-Square=0.000).

3.2.16 Coagulase Test Results for Different Parts of Poultry Samples

Table 3.91 shows the coagulase test results of swabs taken from different parts of poultry samples.

Table 3.91: Coagulase test results of swabs taken from different parts of poultry samples

Part of Chicken		Coagulase			
		Negative (BCGN)	Positive (BCGP)	No Test	Total
FNAS	n	4	0	1	5
	%	80.0%	0.0%	20.0%	100%
FULW	n	24	13	12	49
	%	49.0%	26.5%	24.5%	100%
FURW	n	35	13	6	54
	%	64.8%	24.1%	11.1%	100%
FULT	n	28	10	17	55
	%	50.9%	18.2%	30.9%	100%
FURT	n	42	12	7	61
	%	68.9%	19.7%	11.5%	100%
IEO	n	11	0	34	45
	%	24.4%	0.0%	75.6%	100%
INAS	n	8	4	8	20
	%	40.0%	20.0%	40.0%	100%
Total	n	152	52	85	289
	%	52.6%	18.0%	29.4%	100%

Bacteria coagulase-negative=BCGN; Bacteria coagulase-positive=BCGP; From neck after slaughter=FNAS; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	71.837[a]	12	0.000
Likelihood Ratio	75.725	12	0.000
Linear-by-Linear Association	12.691	1	0.000
No. of Valid Cases	289		

[a]4 cells (19.0%) have expected count less than 5. The minimum expected count is 0.90.

The rates of coagulase-negative (BCGN) test results were very high in all specimens taken from all chicken parts after slaughter, i.e., FNAS (80.0%; 4 out of 5), FULW (49.0%; 24 out of 49), FURW (64.8%; 35 out of 54), FULT (50.9%; 28 out of 55), FURT (68.9%; 42 out of 61), IEO (24.4%; 11 out of 45) and INAS (40.0%; 8 out of 20). By contrast, the rates of coagulase-positive (BCGP) test results were lower for FULW (26.5%; 13 out of 49), FURW

(24.1%; 13 out of 54), FULT (18.2%; 10 out of 55), FURT (19.7%; 12 out of 61) and INAS (20.0%; 4 out of 20), whereas FNAS and IEO did not yield any BCGP rates, but their corresponding no test results were 20.0% (1 out of 5) and 75.6% (34 out of 45), respectively. No test results were also observed in cases of the following parts of chicken specimens analyzed: FNAS (20.0%; 1 out of 5), FULW (24.5%; 12 out of 49), FURW (11.1%; 6 out of 54), FULT (30.9%; 17 out of 55), FURT (11.5%; 7 out of 61) and INAS (40.0%; 8 out of 20). Overall, the BCGN rate was higher (52.6%; 152 out of 289) than the BCGP rate (18.0%; 52 out of 289) in the samples tested. The statistics show that the differences between coagulase results for various chicken parts is significant (Chi-Square=0.000).

3.2.17 Bacterial Diagnostic Test Results for Different Parts of Poultry Samples

Table 3.92 shows the bacterial diagnostic test results of swabs taken from different parts of poultry samples. The highest BGN rate was in IEO (80.0%; 36 out of 45), while the lowest BGN rate was in FURW (9.2%; 5 out of 54). The highest rate of *S. aureus* was in FURW and FULW with counts of 27.8% (15 out of 54) and 26.5% (13 out of 49), while the lowest rate of *S. aureus* was in IEO with a count of 2.2% (1 out of 45). The highest rate of SCN was in FNAS (80.0%; 4 out of 5), while the lowest rate of SCN was in IEO (15.6%; 7 out of 45). The highest rates of *Streptococcus* were in FULW (6.1%; 3 out of 49), IEO (2.2%; 1 out of 45) and FULT (1.8%; 1 out of 55). Overall, the SCN rate (49.8%; 144 out of 289) exceeded both the BGN (30.1%; 87 out of 289) and *S. aureus* (18.0%; 52 out of 289) rates, whereas the *Streptococcus* rate (2.1%; 6 out of 289) was the lowest. The statistics show that the differences between bacterial diagnostic test results for various chicken parts is significant (Chi-Square=0.000).

We found in our study that the rate of BGN in IEO was 80%, which agrees with a study by Buňková et al. (2010), that reported 88% *Enterobacteriaceae*, genus *Aeromonas* bacteria and genus *Pseudomonas* from chicken skin. In addition, we found *Streptococci* in FULW at a rate of 6.1%, which harmonizes with observations made by Barnes et al. (1972) that the ability of

48 strains of anaerobic bacteria exemplify 20 various groups of Gram-negative and Gram-positive *Streptococci* isolated from poultry caeca.

Table 3.92: Bacterial diagnostic test results of swabs taken from different parts of poultry samples

Part of Chicken		Bacterial Diagnostic Test				
		BGN	*S. aureus*	SCN	*Streptococcus*	Total
FNAS	n	1	0	4	0	5
	%	20.0%	0.0%	80.0%	0.0%	100%
FULW	n	10	13	23	3	49
	%	20.4%	26.5%	46.9%	6.1%	100%
FURW	n	5	15	33	1	54
	%	9.2%	27.8%	61.1%	1.9%	100%
FULT	n	16	8	30	1	55
	%	29.1%	14.5%	54.5%	1.8%	100%
FURT	n	11	12	38	0	61
	%	18.0%	19.7%	62.3%	0.0%	100%
IEO	n	36	1	7	1	45
	%	80.0%	2.2%	15.6%	2.2%	100%
INAS	n	8	3	9	0	20
	%	40.0%	15.0%	45.0%	0.0%	100%
Total	n	87	52	144	6	289
	%	30.1%	18.0%	49.8%	2.1%	100%

Bacteria Gram-negative (*Enterobacteriaceae+Pseudomonas*)=BGN; *S. aureus=Staphylococcus aureus*-coagulase positive; *Staphylococcus aureus*-coagulase negative=SCN; From neck after slaughter=FNAS; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	83.779[a]	18	0.000
Likelihood Ratio	83.668	18	0.000
Linear-by-Linear Association	21.082	1	0.000
No. of Valid Cases	289		

[a]11 cells (39.3%) have expected count less than 5. The minimum expected count is 0.10.

Furthermore, our findings agree with a study by Zhao et al. (2001) who documented 25 (3.0%) of the meat samples positive for *Salmonella* and 11.9% of the turkey samples were positive for *E. coli* in samples sourced from 4 supermarkets. Significant variation in the bacterial contamination rates were observed for the 4 supermarket chains. Moreover, the prevalence of microorganisms correlated with resistance to clinically important antibiotics such as erythromycin and oxacillin (Waters et al., 2011).

This agrees with a study by Pesavento et al. (2007) in which *S. aureus* isolated from 42 of the 176 samples of raw meat (poultry, beef and pork) did not express resistance to methicillin. Sallam et al. (2015) suggested that retail chicken might be a pollution source for transmission of resistant *S. aureus*.

Moreover, this agree with a study by Álvarez-Fernández et al. (2012) which found *Enterobacteriaceae* (*Salmonella*) in 55% of the samples in 1993 and 12.4% in 2006 from wings, necks, carcasses and legs of slaughtered chickens. Also, in our study, we found *S. aureus* in FURW (27.8%) and FULW (26.5%), which agrees with a study by Owuna et al. (2015) who reported 29 (72.5%) of *S. aureus* from 40 samples of chicken meat.

Furthermore, we found Gram-negative bacteria in IEO (80.0%), FULT (29.1%), FULW (20.4%) and FNAS (20.0%) and INAS (40.0%) which is in agreement with a study by Guo et al. (2010) who reported *E. coli*, *K. pneumoniae*, *Proteus mirabilis* and *P. aeruginosa* isolates at rates of 53.6%, 25.8%, 3.8% and 90.2%, respectively. Moreover, we found *Streptococcus* in FULW, IEO, FURW and FULT at rates of 6.1%, 2.2%, 1.9%, and 1.8%, respectively.

These rates agree with a study by Castro et al. (2018) which observed *Streptococcus suis* at rates of 56% of from swine and poultry. Amer et al. (2017) observed *Streptococcus* and *Staphylococcus* and 7 Gram-negative bacteria, including *Salmonella spp*., *E. coli*, *Proteus spp*, *Pseudomonas spp* and *Klebsiella* from 360 chicken samples, albeit that 160 were dead in their shells and the other 200 were day-old chicks).

3.2.18 Mueller-Hinton Agar Culture Test Results for Different Parts of Poultry Samples

Table 3.93 shows the Mueller-Hinton agar (MHA) culture test results of swabs taken from different parts of poultry samples. MHA-positive test results were observed in FULW (32.7%; 16 out of 49), FURW (27.8%; 15 out of 54), FULT and FURT (16.4%; 9 out of 55 and 16.4%; 10 out of 61, respectievly), IEO (4.4%; 2 out of 45) and INAS (20.0%; 4 out of 20).

MHA-no-test results were high in all samples tested, i.e., FNAS (100%; 5 out of 5), FULW (67.3%; 33 out of 49), FURW (72.2%; 39 out of 54), FULT and FURT (83.6%; 46 out of 55 and 51 out of 61, respectively), IEO (95.6%; 43 out of 45) and INAS (80.0%; 16 out of 20).

Overall, the MHA-no-test results (80.6%; 233 out of 289) exceeded the MHA-positive test results (19.4%; 56 out of 289). The statistics show that the differences between the MHA test results for various chicken parts is significant (Chi-Square=0.012).

Table 3.93: Mueller-Hinton agar culture test results of swabs taken from different parts of poultry samples

Part of Chicken		Mueller-Hinton Agar Cultures		
		Positive	No Test	Total
FNAS	n	0	5	5
	%	0.0%	100%	100%
FULW	n	16	33	49
	%	32.7%	67.3%	100%
FURW	n	15	39	54
	%	27.8%	72.2%	100%
FULT	n	9	46	55
	%	16.4%	83.6%	100%
FURT	n	10	51	61
	%	16.4%	83.6%	100%
IEO	n	2	43	45
	%	4.4%	95.6%	100%
INAS	n	4	16	20
	%	20.0%	80.0%	100%
Total	n	56	233	289
	%	19.4%	80.6%	100%

From neck after slaughter=FNAS; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.246[a]	6	0.012
Likelihood Ratio	18.624	6	0.005
Linear-by-Linear Association	7.840	1	0.005
No. of Valid Cases	289		

[a]3 cells (21.4%) have expected count less than 5. The minimum expected count is 0.97.

3.2.19 *S. aureus* Antibiotic-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

3.2.19.1 *S. aureus* Oxacillin-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.94 shows the *S. aureus* oxacillin-resistant and-sensitive profiles in different parts of poultry samples.

Table 3.94: *S. aureus* oxacillin-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Oxacillin		
		OXRSA	OXSSA	Total
FULW	n	0	13	13
	%	0.0%	100%	100%
FURW	n	0	13	13
	%	0.0%	100%	100%
FULT	n	0	8	8
	%	0.0%	100%	100%
FURT	n	0	10	10
	%	0.0%	100%	100%
IEO	n	0	1	1
	%	0.0%	100%	100%
INAS	n	0	3	3
	%	0.0%	100%	100%
Total	n	0	48	48
	%	0.0%	100%	100%

OXRSA=oxacillin-resistant *S. aureus*; OXSSA=oxacillin-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	[a]		
Likelihood Ratio			
Linear-by-Linear Association			
No. of Valid Cases	48		

[a]No statistics are computed because Oxacillin is a constant.

All parts of the chicken tested 100% positive for OXSSA. No OXRSA could be demonstrated in all parts of the chicken. Our result does not agree with a study by Lee (2006) which detected the MRSA carrying mecA gene by PCR testing of 19 specimens. However, our results agree with a study by Pesavento et al. (2007). in which 42 isolates of *S. aureus* of the 176 samples

of raw meat (poultry, beef and pork) showed no resistance to methicillin. By contrast, Gundogan et al. (2005) reported a 67.5% MRSA rate, while Jamali et al. (2015) observed a 16.2% OXRSA rate in isolates from dairy milk products and Ge et al. (2017) who documented that 27.9% of *S. aureus* strains were MRSA, representing 1.9% of the total samples of retail meats from eight US suppliers. A study by Darwish et al. (2018) found MRSA was 78.57% as validated by coagulase-positive test results obtained from chicken breast meat, wings, giblets and liver. Moreover, Abdalrahman et al. (2015) illustrated a 0.9% MRSA rate in a total of 167 samples from the chicken and turkey. Furthemore, Sallam et al. (2015) found resistance to penicillin (93.4%), ampicillin (88.8%) and cloxacillin (83.3%) correlated with a 38% MRSA rate in 200 samples analyzed by PCR for the *mecA* gene. The same authors found 288 MRSA-positive strains from chicken samples. Also, Lee (2003) showed resistance to ampicillin and oxacillin in 15 (3 isolates were from chicken and 12 were from dairy cows) of the 28 specimens that tested PCR-positive for the *mecA* gene.

3.2.19.2 *S. aureus* Vancomycin-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.95 shows the *S. aureus* vancomycin-resistant and-sensitive profiles in different parts of poultry samples. VRSA was detected in FULT (12.5%; 1 out of 8) and FURT (10%; 1 out of 10). High rates of VSSA were detected in all parts of the chicken after slaughter, i.e., FULW and FURW (100%; 13 out of 13), FULT (87.5%; 7 out of 8), FURT (90.0%; 9 out of 10), IEO (100%; 1 out of 1) and INAS (100%; 3 out of 3). The statistics show that the differences between the VRSA and VSSA test results for various chicken parts is significant (Chi-Square=0.616). Our VSSA results agree with the study conducted by Gundogan et al. (2005) in which it was shown that every strain was sensitive to vancomycin. Furthemore our result agrees with the findings of the study by Nworie et al. (2017) in which it was found that the rate of resistance of *S. aureus* to vancomycin was 0.0%) in isolates from chickens. Moreover, our study corroborates previous assertions that *S. aureus* strains are highly susceptible to vancomycin (Lee, 2003).

Table 3.95: *S. aureus* vancomycin-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Vancomycin		
		VRSA	VSSA	Total
FULW	n	0	13	13
	%	0.0%	100%	100%
FURW	n	0	13	13
	%	0.0%	100%	100%
FULT	n	1	7	8
	%	12.5%	87.5%	100%
FURT	n	1	9	10
	%	10.0%	90.0%	100%
IEO	n	0	1	1
	%	0.0%	100%	100%
INAS	n	0	3	3
	%	0.0%	100%	100%
Total	n	2	46	48
	%	4.2%	95.8%	100%

VRSA=vancomycin-resistant *S. aureus*; VSSA=oxacillin-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.548[a]	5	0.616
Likelihood Ratio	4.098	5	0.535
Linear-by-Linear Association	0.757	1	0.384
No. of Valid Cases	48		

[a]8 cells (66.7%) have expected count less than 5. The minimum expected count is 0.04.

However, Otalu et al. (2011), observed resistance of *S. aureus* to vancomycin in 8 (61.5%) of the isolates from birds and chickens after slaughter. Likewise, Sallam et al. (2015) detected resistance to vancomycin in 5.9% of MRSA-positive strains isolated from 288 chicken samples. Furthemore our results agree with the findings of Ahmed et al. (2010) in which 86 (51%) of isolates were confirmed as MRSA, and that 23 (27%) of the isolates were resistant to vancomycin, but none of the isolates were vancomycin-resistant.

3.2.19.3 *S. aureus* Tetracycline-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.96 shows the *S. aureus* tetracycline-resistant and-sensitive profiles in different parts of

poultry samples.

Table 3.96: *S. aureus* tetracycline-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Tetracycline			
		TRSA	TSSA	TISSA	Total
FULW	n	8	5	0	13
	%	61.5%	38.5%	0.0%	100%
FURW	n	8	5	0	13
	%	61.5%	38.5%	0.0%	100%
FULT	n	3	4	1	8
	%	37.5%	50.0%	12.5%	100%
FURT	n	4	2	4	10
	%	40.0%	20.0%	40.0%	100%
IEO	n	1	0	0	1
	%	100%	0.0%	0.0%	100%
INAS	n	2	1	0	3
	%	66.7%	33.3%	0.0%	100%
Total	n	26	17	5	48
	%	54.2%	35.4%	10.4%	100%

TRSA=tetracycline-resistant *S. aureus*; TSSA=tetracycline-sensitive *S. aureus*; TISSA=tetracycline-intermediate-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.653[a]	10	0.145
Likelihood Ratio	14.638	10	0.146
Linear-by-Linear Association	1.433	1	0.231
No. of Valid Cases	48		

[a]15 cells (83.3%) have expected count less than 5. The minimum expected count is 0.10.

High levels of TRSA was detected in all parts of chicken samples tested, i.e., FULW and FURW (61.5%; 8 out of 13), FULT (37.5%; 3 out of 8), FURT (40%; 4 out of 10), IEO (100%; 1 out of 1) and INAS (66.7%; 2 out of 3). Overall, the TRSA rate (54.2%; 26 out of 48) was also greater than rates for TSSA (35.4%; 17 out of 48) and TISSA (10.4%; 5 out of 48). TSSA rates were also high in all chicken parts tested, i.e., FULW and FURW (38.5%; 5 out of 13), FULT (50%; 4 out of 8), FURT (20.0%; 2 out of 10) and INAS (33.3%; 1 out of 3), except in IEO for which no TSSA could be demonstrated. TISSA was not detected in FULW, FURW, IEO and INAS, but were found at high levels in FULT (12.5%; 1 out of 8) and FURT (40.0%;

4 out of 10). The statistics show that the differences between the TRSA, TSSA and TISSA test results for various chicken parts is not significant (Chi-Square=0.145). The high rates of TRSA observed in our study are consistent with those published by Jamali et al. (2015) in that the TRSA rate was 56.1% in *S. aureus* isolates from dairy milk products. Similarly, Kim et al. (2018) found that the rate of resistance of *S. aureus* to tetracycline was 7 (21.9%) in isolates from the chickens, and Nworie et al. (2017) reported that the rate of resistance of *S. aureus* to tetracycline was 113 (45.7%) in the isolates from the chickens tested in their study. In addition, a study by Otalu et al. (2011) found resistance to *S. aureus* by tetracycline was 8 (61.5%) of the isolates of birds and chicken after slaughter. Finally, a study by Sallam et al. (2015) found resistance to tetracycline had a count of 68.4% of the 288 MRSA-positive strains isolated from chicken samples.

3.2.19.4 *S. aureus* Erythromycin-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.97 shows the *S. aureus* erythromycin-resistant and-sensitive profiles in different parts of poultry samples. The ERSA rate was high in all chicken parts tested, i.e., FULW and FURW (76.9%; 10 out of 13), FULT (50.0%; 4 out of 8), FURT (70.0%; 7 out of 10), IEO (100%; 1 out of 1) and INAS (66.7%; 2 out of 3). Overall, ERSA rates (70.8%; 34 out of 48) exceeded those of EISSA (27.1%; 13 out of 48) and ESSA (2.1%; 1 out of 48). Rates of EISSA were also high in all parts of the chicken, i.e., FULW and FURW (23.1%; 3 out of 13), FULT (50.0%; 4 out of 8), FURT (20%; 2 out of 10) and INAS (33.3%; 1 out of 3), but for IEO no EISSA was detectable. ESSA was not generally detectable in any part of the chicken, except in FURT (10.0%; 1 out of 10). The statistics show that the differences between the ERSA, ESSA and EISSA test results for various chicken parts is not significant (Chi-Square=0.748). We found 10.0% ESSA in FURT which is lower than that (41.4%) reported by Owuna et al. (2015). However, our ERSA results align with those reported by the previous authors. Aarestrup (2000) demonstrated an ERSA of 24% out of a total of 118 Staphylococcus spp isolated from poultry samples in Denmark.

Table 3.97: *S. aureus* erythromycin-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Erythromycin			
		ERSA	EISSA	ESSA	Total
FULW	n	10	3	0	13
	%	76.9%	23.1%	0.0%	100%
FURW	n	10	3	0	13
	%	76.9%	23.1%	0.0%	100%
FULT	n	4	4	0	8
	%	50.0%	50.0%	0.0%	100%
FURT	n	7	2	1	10
	%	70.0%	20.0%	10.0%	100%
IEO	n	1	0	0	1
	%	100%	0.0%	0.0%	100%
INAS	n	2	1	0	3
	%	66.7%	33.3%	0.0%	100%
Total	n	34	13	1	48
	%	70.8%	27.1%	2.1%	100%

ERSA=erythromycin-resistant *S. aureus*; EISSA=erythromycin-intermediate-sensitive *S. aureus*; ESSA=erythromycin-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.759[a]	10	0.748
Likelihood Ratio	6.118	10	0.805
Linear-by-Linear Association	0.505	1	0.477
No. of Valid Cases	48		

[a]14 cells (77.8%) have expected count less than 5. The minimum expected count is 0.02.

Additionally, the 7.5%, 18.80% and 19.4% ERSA rates documented for isolates from the chickens by Gundogan et al. (2005), Kim et al. (2018) and Nworie et al. (2017), respectively, are much lower than our findings. However, our ERSA rates agree with the reported 73.6% of 288 MRSA-positive strains isolated from the chicken samples (Sallam et al., 2015).

3.2.19.5 *S. aureus* Gentamicin-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.98 shows the *S. aureus* gentamicin-resistant and-sensitive profiles in different parts of poultry samples. GRSA was observed in FULW (7.7%; 1 out of 13) and FURW (15.4%; 2 out of 13), but not in the other chicken parts tested.

Table 3.98: *S. aureus* gentamicin-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Gentamicin		
		GRSA	GSSA	Total
FULW	n	1	12	13
	%	7.7%	92.3%	100%
FURW	n	2	11	13
	%	15.4%	84.6%	100%
FULT	n	0	8	8
	%	0.0%	100%	100%
FURT	n	0	10	10
	%	0.0%	100%	100%
IEO	n	0	1	1
	%	0.0%	100%	100%
INAS	n	0	3	3
	%	0.0%	100%	100%
Total	n	3	45	48
	%	6.2%	93.8%	100%

GRSA=gentamicin-resistant *S. aureus*; GSSA=gentamicin-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.364[a]	5	0.644
Likelihood Ratio	4.231	5	0.517
Linear-by-Linear Association	1.392	1	0.238
No. of Valid Cases	48		

[a]8 cells (66.7%) have expected count less than 5. The minimum expected count is 0.06.

The GSSA rates were very high in all parts of the chicken samples tested, i.e., FULW (92.3%; 12 out of 13), FURW (84.6%; 11 out of 13), FULT, FURT, IEO and INAS (100%; 8 out of 8, 100%; 10 out of 10, 100%; 1 out of 1 and 100%; 3 out of 3, respectively. Overall the GSSA rate (93.8%; 45 out of 48) exceeded that of the GRSA (6.2%; 3 out of 48) 15-fold. The statistics show that the differences between the GRSA and GSSA test results for various chicken parts is not significant (Chi-Square=0.644). The high GSSA rates observed in this study compares favourably with the rate of 82.8% reported previously by Owuna et al. (2015). Additionlly, our GRSA results agrees with the findings of Kim et al. (2018) and Otalu et al. (2011) in which it was found that the rates of *S. aureus* resistance to gentamicin was 5.3% and 38.5%, respectively, in isolates from the chickens after slaughter. Our results also corroborate the study

by Sallam et al. (2015) in which it was found that resistance to gentamicin was 24.3% of 288 MRSA-positive strains isolated from chicken samples.

3.2.19.6 *S. aureus* Kanamycin-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.99 shows the *S. aureus* kanamycin-resistant and-sensitive profiles in different parts of poultry samples.

Table 3.99: *S. aureus* kanamycin-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Kanamycin		
		KRSA	KSSA	Total
FULW	n	4	9	13
	%	30.8%	69.2%	100%
FURW	n	6	7	13
	%	46.2%	53.8%	100%
FULT	n	0	8	8
	%	0.0%	100%	100%
FURT	n	3	7	10
	%	30.0%	70.0%	100%
IEO	n	0	1	1
	%	0.0%	100%	100%
INAS	n	0	3	3
	%	0.0%	100%	100%
Total	n	13	35	48
	%	27.1%	72.9%	100%

KRSA=kanamycin-resistant *S. aureus*; KSSA=kanamycin-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.984[a]	5	0.222
Likelihood Ratio	9.862	5	0.079
Linear-by-Linear Association	1.874	1	0.171
No. of Valid Cases	48		

[a]8 cells (66.7%) have expected count less than 5. The minimum expected count is 0.27.

The KRSA rates were consistently lower than the KSSA rates in all parts of the chicken tested, i.e., in FULW (30.8%; 4 out of 13 vs 69.2%; 9 out of 13), FURW (46.2%; 6 out of 13 vs 53.8%; 7 out of 13), FULT (0% vs 100%; 8 out of 8), FURT (30.0%; 3 out of 10 vs 70.0%; 7 out of

10), IEO (0% vs 100%; 3 out of 3). Likewise, the overall KRSA rate was 2.7-fold less (27.1%; 13 out of 48) than the KSSA rate (72.9%; 35 out of 48). The statistics show that the differences between the KRSA and KSSA test results for various chicken parts is not significant (Chi-Square=0.222). Our observed KRSA rates, however, exceeded the 4% rate of resistance of *S. aureus* to kanamycin in isolates from dairy milk products (Jamali et al., 2015). In our study we found a KRSA rate of 46.2% in FURW which is in agreement with the findings of the study by Kim et al. (2018), viz., the rate of resistance of *S. aureus* to kanamycin was 48.5% in chicken samples tested.

3.2.19.7 *S. aureus* Streptomycin-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.100 shows the *S. aureus* streptomycin-resistant and-sensitive profiles in different parts of poultry samples. SRSA was detected in FULW (23.1%; 3 out of 13), FURW (7.7%; 1 out of 13) and INAS (33.3%; 1 out of 3), but not in the other parts of the chicken samples tested. By comparison, SSSA was detected in high levels in all samples, i.e., FULW (76.9%; 10 out of 13), FURW (92.3%; 12 out of 13), FULT (100%; 8 out of 8), FURT (100%; 10 out of 10), IEO (100%; 1 out of 1) and INAS (66.7%; 2 out of 3). Overall, the SSSA rate (89.6%; 43 out of 48) was 8.6-fold higher than the SRSA rate (10.4%; 5 out of 48). The statistics show that the differences between the SRSA and SSSA test results for various chicken parts is not significant (Chi-Square=0.284). Our rates of SRSA agree with those reported for MRSA-positive strains identified in chicken samples (Owuna et al., 2015; Sallam et al., 2015).

3.2.19.8 *S. aureus* Amikacin-Resistant and-Sensitive Profiles in Different Parts of Poultry Samples

Table 3.101 shows the *S. aureus* amikacin-resistant and-sensitive profiles in different parts of poultry samples. No ARSA was detected in chicken samples, but ASSA was 100% positive in all parts of the chicken tested, Thus, all the *S. aureus* strains were sensitive to amikacin, which agrees with a study by Lee (2003) which found all strains were also susceptible to amikacin in

the 15 isolates (3 were from chickens and 12 were from dairy cows).

Table 3.100: *S. aureus* streptomycin-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Streptomycin		
		SRSA	SSSA	Total
FULW	n	3	10	13
	%	23.1%	76.9%	100%
FURW	n	1	12	13
	%	7.7%	92.3%	100%
FULT	n	0	8	8
	%	0.0%	100%	100%
FURT	n	0	10	10
	%	0.0%	100%	100%
IEO	n	0	1	1
	%	0.0%	100%	100%
INAS	n	1	2	3
	%	33.3%	66.7%	100%
Total	n	5	43	48
	%	10.4%	89.6%	100%

SRSA=streptomycin-resistant *S. aureus*; SSSA=streptomycin-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.234[a]	5	0.284
Likelihood Ratio	7.162	5	0.209
Linear-by-Linear Association	0.477	1	0.490
No. of Valid Cases	48		

[a]8 cells (66.7%) have expected count less than 5. The minimum expected count is 0.10.

In addition, in a study by Otalu et al. (2011) it was demonstrated that *S. aureus* was susceptible to amikacin in 100% of the isolates of birds and chickens that were slaughtered. Moreover, the latter study is consonant with the studies by Lee (2003) and Sallam et al. (2015) who also observed resistance to amikacin at a rate of 34.4% in the 288 MRSA-positive strains isolated from chicken samples. Furthemore in a study by Guo et al. (2010) it was found that *E. coli, K. pneumoniae, Proteus mirabilis* and *P. aeruginosa* isolates had counts of 53.6%, 25.8% and 3.8%, respectively, the majority (90.2%) of which were sensitive to amikacin.

Table 3.101: *S. aureus* amikacin-resistant and-sensitive profiles in different parts of poultry samples

Part of Chicken		Amikacin		
		ARSA	ASSA	Total
FULW	n	0	13	13
	%	0.0%	100%	100%
FURW	n	0	13	13
	%	0.0%	100%	100%
FULT	n	0	8	8
	%	0.0%	100%	100%
FURT	n	0	10	10
	%	0.0%	100%	100%
IEO	n	0	1	1
	%	0.0%	100%	100%
INAS	n	0	3	3
	%	0.0%	100%	100%
Total	n	0	48	48
	%	0.0%	100%	100%

ARSA=amikacin-resistant *S. aureus*; ASSA=amikacin-sensitive *S. aureus*; From under left wing=FULW; From under right wing=FURW; From under the left thigh=FULT; From under the right thigh=FURT; Inside external os of the chicken=IEO; Inside neck after slaughter=INAS

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	[a]		
Likelihood Ratio			
Linear-by-Linear Association			
No. of Valid Cases			

[a]No statistics are computed because Amikacin is a constant.

CHAPTER 4

CONCLUSION AND PERSPECTIVES

4.1 The Historical and Modern Continuum of MRSA Infections

Over the last 50 years, methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged from a worldwide nosocomial or health care-associated pathogen (HA-MRSA) into the causative infectious agent of community-acquired MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA) (Chatterjee & Otto, 2013; Chen & Huang, 2018; Gardam, 2000; Murphy et al., 2019; Siddiqui & Koirala, 2019). MRSA was first reported in the United Kingdom in 1961 (Barber, 1961), soon after the introduction of methicillin, and by the mid-1970s had become endemic in many countries (Voss & Doebbeling, 1995).

Oxacillin (Methicillin)-resistant *Staphylococcus aureus* (*S. aureus)* (ORSA or MRSA) has become a significant nosocomial pathogen in many developed and developing countries (Al-Talib et al., 2010; Buzaid et al., 2011; Carvalho et al., 2010; Hetem et al., 2012; Huang et al., 2011; Mine et al., 2011; Moremi et al., 2012; Schweickert et al., 2011; Song et al., 2011; Wang, F. D. et al., 2011). Some types of MRSA have been designated epidemic strains - these are associated with a higher prevalence and have been shown to spread within hospitals, between hospitals, and between countries (Aires De Sousa et al., 2001; Humphreys et al., 1990; Roberts et al., 1998; Saroglou et al., 1980).

The first MRSA isolates expressed so-called heterogeneous phenotypic resistance to oxacillin, signifying that the oxacillin minimum inhibitory concentrations (MICs) for only subpopulations of isolates are high (Mendes et al., 2010; Schweickert et al., 2011; Sola et al., 2011). Progressively, the heterogeneous oxacillin-resistant phenotype was replaced by the homogeneous oxacillin-resistant phenotype, which is characterized by the expression of

oxacillin resistance by all populations (Said-Salim et al., 2005). Initially, early isolates were also resistant to various other drugs, including penicillin, tetracycline and, usually, streptomycin (some strains were also resistant to erythromycin, lincomycin, neomycin, kanamycin and novobiocin). In 1969, the first clinical gentamicin-resistant MRSA (GR-MRSA) strain was isolated (Lacey & Mitchell, 1969) and, by the 1980s, GR-MRSA had become epidemic in Australia, the United States and Europe (Cafferkey et al., 1983). Such GR-MRSA strains were usually resistant to a broad number of other antibiotics, including trimethoprim and, more recently, ciprofloxacin and mupirocin. In addition to increasing multi-antibiotic drug resistance, the overall incidence of MRSA isolation has gradually increased in many countries to present levels of around 30% in Spain, France and Italy (Voss & Doebbeling, 1995) and up to 54% in Japan (Lotsu et al., 1995).

The emergence of new epidemic MRSA strains with increasing susceptibility to antibiotics has been reported by two French hospitals (Aubry-Damon et al., 1997; Lemaitre et al., 1998). These strains were characterized mainly by the unexpected reappearance of heterogeneous resistance to oxacillin, susceptibility to gentamicin, and variable resistance to macrolides, lincosamides, and streptogramin type B antibiotics. However, they remained resistant to tobramycin, which was associated with the presence of the aminoglycoside nucleotidyl transferase (ANT) (Aubry-Damon et al., 1997; Lemaitre et al., 1998). A marked decrease in the use of gentamicin was suspected to be a factor contributing to the emergence of gentamicin-susceptible MRSA (GS-MRSA) from predominantly GR-MRSA populations (Aubry-Damon et al., 1997; Lemaitre et al., 1998). MRSA has become a leading cause of infections in both the community and health care-related settings (Malachowa et al., 2012; Rivero-Perez et al., 2012). Data from the 2004 Centers for Disease Control and Prevention of National Nosocomial Infections Surveillance System indicate that the prevalence of MRSA now exceeds 50% in most hospitals in the United States ("National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004," 2004). Equally alarming is the recent emergence of MRSA strains in the community setting, causing infections that range from

cellulitis with skin abscesses to pneumonia and endocarditis in otherwise healthy individual (Shukla, 2005).

Vancomycin has been the accepted standard of therapy for MRSA infections (Anurag Payasi, 2015; Levine, 2006; Stevens, 2006; Yoon et al., 2014). Newer agents with proven efficacy against MRSA infections (e.g., linezolid, quinupristin-dalfopristin, daptomycin, and tigecycline) are available, but have not been routinely prescribed because of higher drug acquisition costs and/or relative lack of clinical experience compared with vancomycin. However, treatment failures of vancomycin for MRSA infections have increasingly been reported in the literature despite apparent in vitro susceptibility, particularly for strains with a MIC of 2 μg/ml (Moise-Broder et al., 2004; Sakoulas et al., 2004).

In an attempt to reconcile this discrepancy, the Clinical and Laboratory Standards Institute (CLSI) lowered the vancomycin breakpoint for susceptibility from 4 to 2 μg/ml for *S. aureus* in 2006 ("Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI Document M100-S16 (ISBN 1-56238-625-5). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.," 2007; Wootton et al., 2005).

MRSA has emerged as an important pathogen in hospitalized adults in the United States, but reports of MRSA in paediatric patients have been infrequent. A survey conducted among directors of microbiology at all acute care children's hospitals in the United States revealed that although the frequency at which MRSA is isolated from children varies significantly, it is increasing at an alarming rate (Jarvis et al., 1985). Thus, the increased frequency of MRSA isolation in both adult and paediatric patients may be regarded as a significant contributing factor to adult and childhood morbidity and mortality worldwide, and has established a dire need for further studies to identify risk factors for MRSA infections and to develop effective control measures (Jarvis et al., 1985; Mehndiratta & Bhalla, 2012).

4.2 Study Context and Significance

Based on the responses of *Staphylococcus aureus* to antibiotics, the pathogen can be categorized into two types, viz., methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Kirmusaolu, 2017), also recognized as oxacillin-resistant *Staphylococcus aureus* (ORSA). Methicillin resistance in *S. aureus* is defined as an oxacillin minimum inhibitory concentration (MIC) of greater than or equal to 4 µg/ml. MRSA infections can be further divided into hospital-associated (HA-MRSA) infections and community-associated (CA-MRSA) infections.

They differ not only in respect to their clinical features and molecular biology, but also to their antibiotic susceptibility (Foster, 2017; Giesbrecht et al., 1998; Kirmusaolu, 2017; Lakhundi & Zhang, 2018; Lowy, 2003; Manara et al., 2018; Peacock & Paterson, 2015; Yılmaz & Aslantaş, 2017). The major pathophysiologic factor for MRSA resistance to ß-lactam antibiotics is the presence of the *mecA* gene sequence, which encodes a transpeptidase, i.e., a penicillin binding protein 2A (PBP2a) that lowers the affinity of the *S. aureus* to bind to ß-lactam antibiotics (Fisher & Mobashery, 2016; Kirmusaolu, 2017; Schito, 2006).

Biofilm infections caused by *S. aureus* infections are particularly challenging in hospitalized and immunosuppressed patients worldwide since they are refractory to antibiotic treatment and resistant to host immune defences (Ahmadrajabi et al., 2017; Alboslemy et al., 2019; Brady et al., 2018; Sultan et al., 2018). Moreover, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is rapidly becoming an emerging threat to public health and establishing the clinical correlations between CA-, HA- and LA-MRSA has become a challenging task to illuminate their genetic diversity and evolution (Abd El-Hamid et al., 2019; Copin et al., 2019; Hogan et al., 2019; Mekonnen et al., 2019; Nagasundaram & Sistla, 2019; Peng et al., 2018; Roberts et al., 2018). Besides, the World Health Organization (WHO) Global Report on Antimicrobial Resistance (AMR), accentuated that antibacterial resistance (ABR) is a major contributor to intensifying the global risk of the spread of *S. aureus* infections,

prolonging morbidity and hospitalization, and adding to economic and social burden of world populations (Chen et al., 2018; Nelson et al., 2018; World Health Organization, 2014). MRSA prevalence constitutes a significant hurdle to successful infection eradication and control in hospitals in Libya, and thus serves as an ominous signal to institute a comprehensive surveillance and prevention programme in the country to reduce MRSA and other antimicrobial-resistant pathogens, as observed in Misurata hospitals, Libya (Buzaid et al., 2011).

This study was aimed at identifying antibiotic susceptibilities that specifically target MRSA strains isolated from stored patient samples in selected hospital departments and laboratories in Libya. In addition, the study sought to analyze the extent of MRSA contamination of poultry samples obtained from various markets in Misurata.

4.3 Conclusions and Perspectives

In this study, 657 patient samples were collected from blood culture, cerebrospinal fluid (CSF), eye swab, lung swab, semen, sputum, stool, swabs, swabs from operation, throat swabs, urine and wound swabs. The swab samples were taken at departments of the three main hospitals (ICU, Inpatients, Newborns and Outpatients), as well as at a private clinical laboratory, all located in Misurata, Libya. Also, 361 swabs from chicken samples were collected from five locations of which 72 showed no bacterial growth, while 289 showed bacterial growth.

Antimicrobial susceptibility testing of the MRSA and MSSA patient isolates was performed using the Kirby-Bauer disc diffusion method. Polymerase chain reaction (PCR) was performed on the patient bacterial isolates to test for target genes *mecA1* and *mecA2* to identify *Staphylococcus aureus* resistance. The oxacillin disk was a reference method for the identification of MRSA strains. A total of 37 MRSA from 73 strains of *S. aureus* were identified. In patient samples, antibiotic-resistant *S. aureus* rates were as follows: oxacillin-resistant S. aureus (OXRSA; 51.7%), vancomycin-resistant *S. aureus* (VRSA; 1.7%),

erythromycin-resistant *S. aureus* (ERSA; 76.7%), gentamicin-resistant *S. aureus* (GRSA; 11.7%), kanamycin-resistant *S. aureus* (KRSA; 50%), streptomycin-resistant *S. aureus* (SRSA; 18.3%), amikacin-resistant *S. aureus* (ARSA; 11.7%), while the PCR+ (expression of the *mecA1* and *mecA2* genes) rate was 96.7%. By contrast, antibiotic-sensitive *S. aureus* rates were as follows: oxacillin-sensitive *S. aureus* (OXSSA; 48.3%), vancomycin-resistant *S. aureus* (VSSA; 98.3%), erythromycin-sensitive *S. aureus* (ESSA; 21.6%), gentamicin-sensitive *S. aureus* (GSSA; 83.3%), kanamycin-sensitive *S. aureus* (KSSA; 50%), streptomycin-sensitive *S. aureus* (SSSA; 81.7%), amikacin-sensitive *S. aureus* (ASSA; 86.7%), while the PCR- rate was 6.7%.

Of a total of 361 poultry swabs, 19.94% (n=72) showed no bacterial growth, while 80.06% (n=289) showed bacterial growth. Of the 48 samples tested for *S. aureus* antibiotic sensitivity, all were sensitive to oxacillin (100%), whereas 96% were sensitive to vancomycin, 35.4% were sensitive to tetracycline and 10.4% showed intermediate sensitivity to tetracycline, 94% were sensitive to gentamicin, 73% to kanamycin, 90% to streptomycin and 100% to amikacin. *S. aureus* antibiotic-resistance were not observed for oxacillin and amikacin, but for vancomycin (4%), tetracycline (54.5%), erythromycin (71%), gentamicin (6%), kanamycin (27%) and streptomycin (10%).

It is clear from the results of this study that the patient MRSA resistance rate to vancomycin, streptomycin, gentamicin and amikacin was relatively low, although MRSA was highly resistant to oxacillin, erythromycin and kanamycin. MRSA was highly susceptible to vancomycin, gentamicin, streptomycin and amikacin. Equally evident also is finding that MRSA in poultry samples showed no resistance to oxacillin and amikacin, but high sensitivity to vancomycin, gentamicin, kanamycin and streptomycin, whereas a high degree of resistance was exhibited towards tetracycline. The extensive use of antibiotics in health care and the food supply chain is considered as the major driving force behind the emergence of drug-resistant bacteria such as *S. aureus*, that are highly adapted to propagate in patients and spread

horizontally to other bacteria and thus to animals and humans (Abebe et al., 2016; Admassie, 2018; Bitrus et al., 2017; DeLeo & Chambers, 2009; Holden et al., 2013; Jamrozy et al., 2017; Lindsay, 2014b; Wu et al.). *Staphylococcus aureus*, methicillin-resistant as well as vancomycin intermediate and resistant strains of the bacteria have been placed on the global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics (World Health Organization, 2017).

The present study highlights a significant spread of MRSA in the city of Misurata in Libya, and underscores the need for health education and promotion insofar as infectious diseases and prudent antibiotic therapies are concerned. Such initiatives will strengthen public awareness as well as influence the implementation of control and preventive measures to infections caused by MRSA. It is hoped that the data obtained in this thesis will represent the scale of the many endeavours and conscious initiatives that are currently being embarked on to understand the molecular epidemiology of *S. aureus* isolates, not only in terms of the persistence and spread of CA-MRSA, HA-MRSA and LA-MRSA, but also to focus awareness on rational antibiotic prescription policies and procedures, as well as prevention and control of HA-, CA- and LA-MRSA transmission in Libya.

4.4 Limitations

The present study has several limitations. The sample size was small as it was limited to hospitals and laboratories and a few poultry markets, and, as such the results cannot be interpreted to reflect the current state of bacterial and *S. aureus* (MRSA) infections in Libya. The study design was not optimized for proper evaluation of MRSA genes by PCR. Larger sample sizes from different hospitals would bolster the external validity of our findings.

4.5 Recommendations

The following recommendations emanate from this study and includes a concise synthesis of recent suggestions and ideas from the literature, as well as directives provided by the World

Health Organization with regard to the global coordination in the fight against antibiotic-resistant bacteria, including MRSA.

- Routine surveillance of MRSA colonization in hospitalized as well as non-hospitalized patients is recommended.

- MRSA is a food-borne pathogen and therefore its detection in food products such as meat, poultry, milk, cheese and diary products should be stepped-up.

- Community contacts are needed to assist public health professionals to better understand the epidemiology and transmission of MRSA and curb its spread and proliferation in Libya (Khemiri et al., 2017; Munch et al., 2017).

- *S. aureus* can survive on inanimate objects for prolonged periods and can easily spread through contaminated fomites. Therefore, objects such as gloves, masks, surgical instruments, attire and blankets are potential reservoirs for MRSA transmission and should be considered in decontamination practices and future study designs (Hogan et al., 2015; Mohamed Ali et al., 2014; Zorgani, A. et al., 2015).

- Household environments and pets are reservoirs of MRSA. Therefore, household member MRSA colonization burden reflects environmental MRSA contamination. Longitudinal studies will improve our understanding of factors associated with *Staphylococcus aureus* environmental surface and pet colonization in households of children with CA-MRSA infection (Hogan et al., 2019).

- Genotyping of LA-MRSA isolates will enhance decontamination strategies and limit infection of livestock farmers and decrease the prevalence of MRSA in animal and food products (Abd El-Hamid et al., 2019; Becker et al., 2017; Fertner et al., 2019; Logue & Andreasen, 2018; Murra et al., 2019; Sorensen et al., 2018).

- Recently, the WHO global priority pathogens list (global PPL) panel pointed out the

lack of surveillance data on livestock and food, highlighting the need for coordination between human and animal surveillance systems. Inaccurate or incomplete surveillance data delay translational research on the antibiotic resistance threat and reduce the effectiveness of the "One Health" approach to limit the spread of resistance (World Health Organization, 2017).

- Although a relatively substantive database presently exists in Libya, there is still a need for periodic - roughly every three months - surveillance studies that should be initiated (Ali et al., 2014; Ghenghesh et al., 2013; Sifaw Ghenghesh et al., 2013; Wareg et al., 2014).

- A dedicated MRSA research centre should be established that would collaborate with the universities and medical centres.

- MRSA should be examined in the noses of all workers in hospitals (Al-haddad et al., 2014). Hence, one should develop and modernize all the hospitals, especially the intensive care units and furnish them with automatic doors and tap water.

- The early detection of MRSA in workers and poultry is essential. Furthermore, administrative follow-ups, the use of disinfectants as well as the development of livestock/poultry quarantine facilities are required.

- In addition, a dedicated clinical microbiology laboratory should be available to receive clinical and demographic characteristics of patients from whom all MRSA strains are isolated. Upon receipt, subcultures of the isolates should be performed immediately, and then stored in a freezer for further specilalized investigations.

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APPENDIX 1

UWC PROJECT REGISTRATION NUMBER

BIOMEDICAL RESEARCH ETHICS COMMITTEE (BMREC (16/2 A)

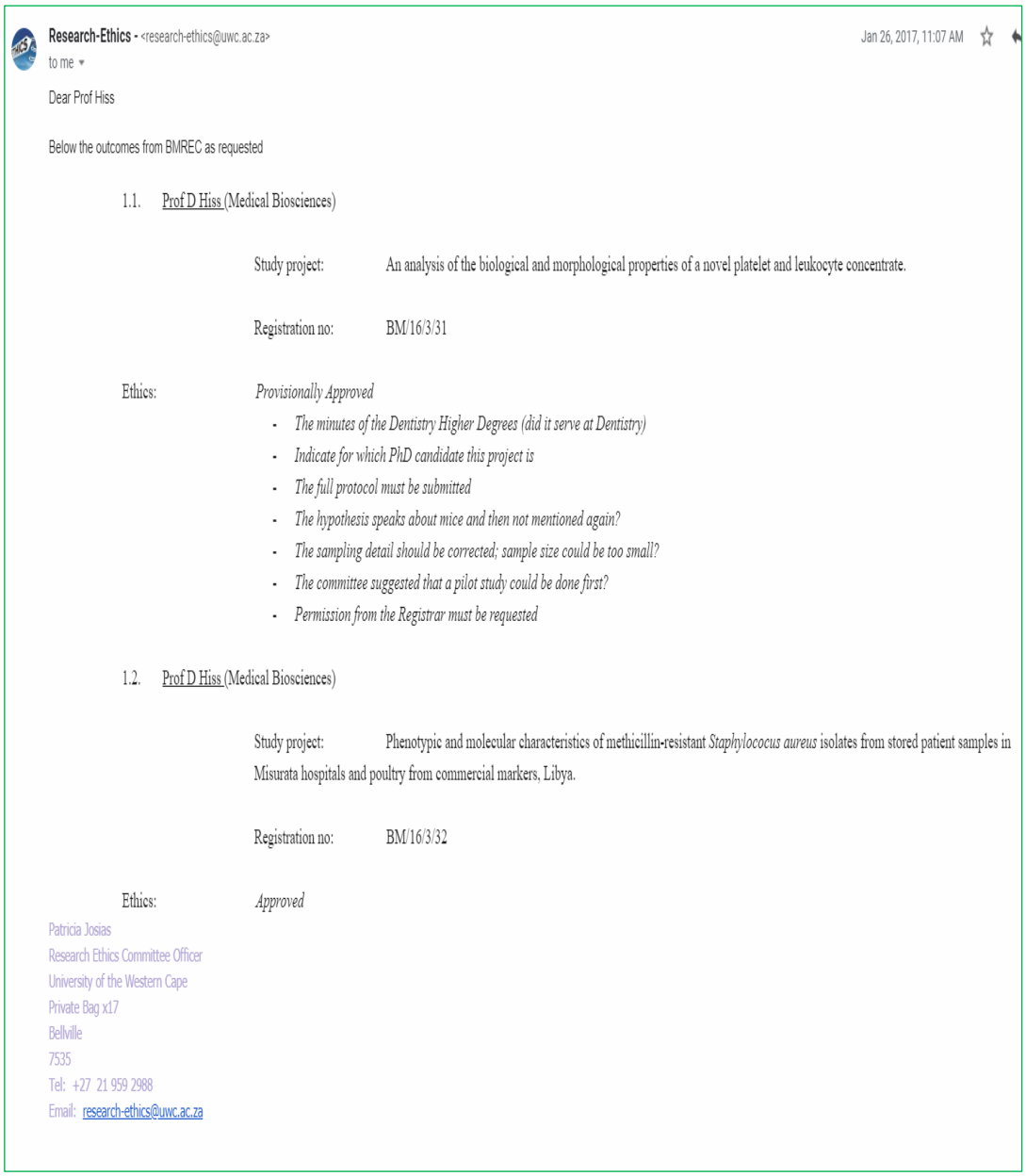
BMREC Application Mr Alhussien Ali Elakrout for a PhD, Prof D Hiss

ScRIRC2016/04/EXCO3.1

Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from stored patient samples in Misurata hospitals and poultry from commercial markets, Libya

APPENDIX 2

UWC BIOMEDICAL RESEARCH ETHICS COMMITTEE CLEARANCE CERTIFICATE



Research-Ethics - <research-ethics@uwc.ac.za> Jan 26, 2017, 11:07 AM

to me

Dear Prof Hiss

Below the outcomes from BMREC as requested

1.1. Prof D Hiss (Medical Biosciences)

Study project: An analysis of the biological and morphological properties of a novel platelet and leukocyte concentrate.

Registration no: BM/16/3/31

Ethics: *Provisionally Approved*

- *The minutes of the Dentistry Higher Degrees (did it serve at Dentistry)*
- *Indicate for which PhD candidate this project is*
- *The full protocol must be submitted*
- *The hypothesis speaks about mice and then not mentioned again?*
- *The sampling detail should be corrected; sample size could be too small?*
- *The committee suggested that a pilot study could be done first?*
- *Permission from the Registrar must be requested*

1.2. Prof D Hiss (Medical Biosciences)

Study project: Phenotypic and molecular characteristics of methicillin-resistant *Staphylococus aureus* isolates from stored patient samples in Misurata hospitals and poultry from commercial markers, Libya.

Registration no: BM/16/3/32

Ethics: *Approved*

Patricia Josias
Research Ethics Committee Officer
University of the Western Cape
Private Bag x17
Bellville
7535
Tel: +27 21 959 2988
Email: research-ethics@uwc.ac.za

APPENDIX 3

ETHICS CLEARANCE CERTIFICATE

EHMAID
For Legal Translation

Translation from Arabic doc.

Assafwa International Hospital

Mr. Alhussien Ali Elakrout

Greeting ..

Referring to your request on date 1/01/2014, concerning the acceptance for taking samples from patients, departments and laboratories in Assafwa International Hospital for your study research , entitled :-

Phenotypic and Molecular Characteristics of Methicillin resistant Staphylococcus Aureus Isolation from Misurata ..

Considering the importance of the scientific research we have actually realized the final treatment results for the use of such antibiotics we are so pleased to accept that you carry on your research knowing that this has no financial, corporal or moral damages.

Best regards ..

Jebril Eljetlawi
Manager of Assafwa International Hospital-Misurata
Signature
12/01/2014

Ring Seal, Reads: Assafwa International Hospital-Misurata

I, Hmaid Yassine , active member of the Libyan Translators, competent in both Arabic & English certify herein that the forgoing is a true and accurate translation of the attached document, rendered this 18th day of March 2015.

عمارات الجامع العالى