

PREVALENCE OF GRAM-NEGATIVE INFECTIONS IN CERVICO-FACIAL SEPSIS

Neil Barnard

Student No. 2381073



A mini-thesis submitted for fulfilling the requirements for the Degree of
Magister Chirurgiae Dentium in the discipline of Maxillo-Facial and Oral
Surgery, Faculty of Dentistry, University of the Western Cape.

Supervisor: Dr A.J. van der Westhuijzen

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KEYWORDS

Cervico-facial

Sepsis

Gram-negative

Bacterial

Sensitivity

Culture

ABBREVIATIONS



NHLS National Health Laboratory Services

MCS Microscopy Culture Sensitivity

TMJ Temporo-Mandibular Joint

ENTCC Enterobacter cloacae subspecies cloacae

TBOHC Tygerberg Oral Health Centre

GLOSSARY

The terms below are defined for the purpose of this study:

Cervico-facial sepsis: An infection that involves the neck and face area.

Pus swab: A sterile swab that is used to collect pus from a site of infection.

Anaerobic bacteria: An organism not requiring oxygen to grow.

Gram-negative organism: Bacteria not retaining the crystal violet stain that is used in the gram-staining method of bacterial differentiation.



ABSTRACT

Prevalence of gram-negative infections in cervico-facial sepsis.

Neil Barnard

MChD (MFOS) mini-thesis, Department of Maxillo-Facial and Oral Surgery, Faculty of Dentistry, University of the Western Cape.

In cervico-facial sepsis a substantial proportion of organisms are normally gram-negative staining of which the majority are anaerobes (Boyanova 2006 and Sanchez 2010). At Tygerberg Academic Hospital and Tygerberg Oral Health Centre it has been found that patients treated for cervico-facial sepsis seldom have gram-negative organisms on culture, although the staining results reported the presence of gram-negative organisms.

The aim of the study was to assess the prevalence of gram-negative staining in a population of patients with cervico-facial sepsis and to determine the number of gram-negative stains that yield gram-negative organisms on culture.

Results indicated that 71 out of a possible 90 pus swabs reported a gram stain. Of those, 48 specimens stained gram-negative and only two of these cultured gram-negative organisms.


Although gram-negative organisms are present on gram stain, microbiological diagnosis of gram-negative organisms on culture was very seldom found at the Maxillofacial and Oral Surgery unit at the Tygerberg Academic Hospital and Tygerberg Oral Health Centre.

DECLARATION

I declare that '**Prevalence of gram-negative infections in cervico-facial sepsis**' is my own work, that it has not been submitted for any degree or examination at any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.



Neil Barnard

Signed: 

June 2019

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The completion of this research project could not have been accomplished without the willingness and guidance of the following individuals:

- Dr AJ van der Westhuijzen, my supervisor, to whom I would like to express my sincere gratitude.
- Dr F Kimmie, for assisting with the research statistics
- Dr Fadi Titinchi, who assisted me with the formatting.



DEDICATION

This thesis is dedicated to my wife, Lize. I thank her for her love and support throughout my years of study.



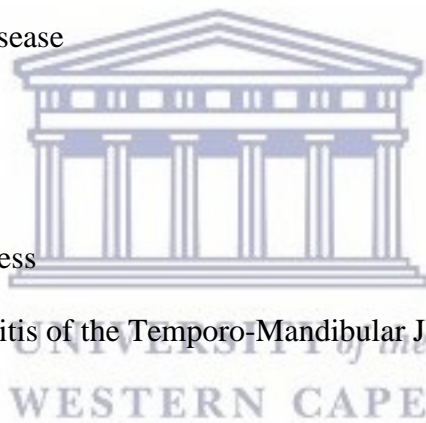
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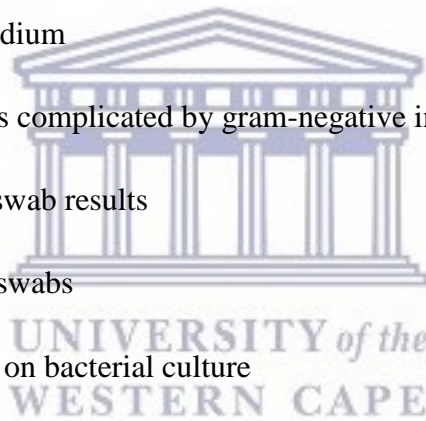
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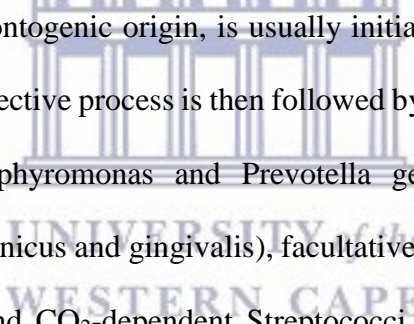
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Chapter 1

INTRODUCTION

Cervico-facial sepsis is managed by Maxillo-Facial and Oral surgeons almost on a daily basis. The management protocol includes emergency management, diagnosis, medical and more than often, surgical intervention. If a drainable collection is present, a sample is sent for microscopy, culture and sensitivity (MCS). The patient is then commenced on empirical antibiotics until the results of microscopy, culture and sensitivity become available (Flynn *et al.* 2003).



Cervicofacial sepsis, from odontogenic origin, is usually initiated by streptococci in the first three days of infection. The infective process is then followed by predominantly gram-negative anaerobic bacteria i.e. Porphyromonas and Prevotella genera (formerly classified as Bacteroides oralis, melaninogenicus and gingivalis), facultative anaerobic gram-positive cocci such as Peptostreptococcus and CO₂-dependent Streptococci such as Streptococcus milleri (Rega *et al.* 2006).

Gill and Scully (1988) conducted a survey in which they highlighted the reluctance of Maxillo-Facial and Oral surgeons in Britain to consider the primary role that anaerobic bacteria play in acute dento-alveolar infections. Subsequent research has also shown that gram-negative anaerobes make up a high percentage of head and neck infections (Boyanova 2006 and Sanchez 2010).

It was noted that many gram-negative stains were reported at Tygerberg Academic Hospital and Tygerberg Oral Health Centre but very few of these were followed by positive cultures. In this context, it was decided to research the matter of pus swab sampling at Tygerberg Academic Hospital with special reference to the prevalence of gram-negative organism in cervico-facial sepsis.

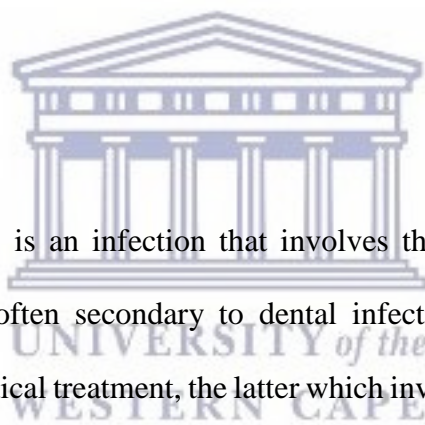


Chapter 2

LITERATURE REVIEW

2.1 Introduction:

The literature review will describe cervico-facial sepsis as well as its methods in microbiological sampling, ideal time interval prior to microbiology laboratory processing, the clinical significance of anaerobic bacteria in cervico-facial sepsis, therapy for cervico-facial sepsis, the spectrum of cervico-facial sepsis and lastly the role of gram-negative anaerobes in human infection.



Bacterial cervico-facial sepsis is an infection that involves the neck and face area. It is a common problem and most often secondary to dental infection. The infection is usually resolved with surgical and medical treatment, the latter which involves antimicrobial treatment. Accurate antimicrobial treatment is based on an accurate antimicrobial diagnosis (J. Byers 2010).

2.2 Microbiology:

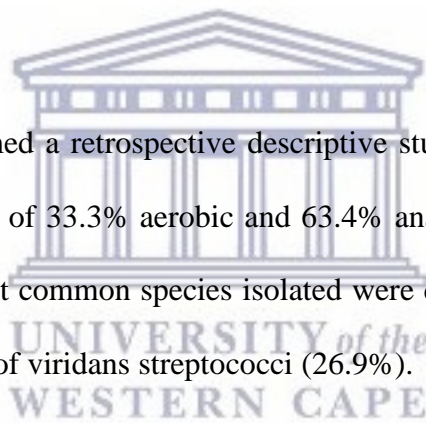
The microflora of the head and neck are typically polymicrobial with an average of two to six isolates per specimen. Anaerobic organisms predominate and occur three to four times more than aerobes. Aerobic streptococci predominate in the first three days of infection and then gram-negative anaerobic bacteria take over the infective process (Rega *et al.* 2006).

Organisms most important in polymicrobial infections are the organisms that are most virulent, exhibit antimicrobial resistance and are present in greatest numbers. In head and neck infection anaerobes fulfil all these features (Finegold 1995).

The anaerobes frequently implicated in cervico-facial infection are the gram-negative bacilli of the *Prevotella* and *Porphyromonas* genera, gram-positive cocci such as *Peptostreptococcus* and also the carbon dioxide dependent Streptococci such as *Streptococcus milleri* (Stefanopoulos 2004).

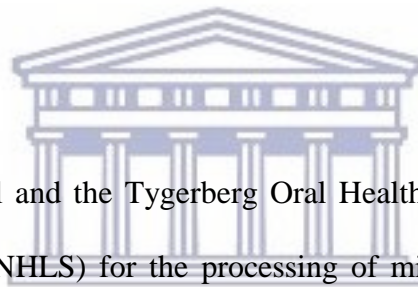
Sanchez *et al.* (2010) performed a retrospective descriptive study on 151 case records. The researcher found a prevalence of 33.3% aerobic and 63.4% anaerobic organisms in cases of cervico-facial sepsis. The most common species isolated were different species of *Prevotella* (26.1%) and different species of viridans streptococci (26.9%).

Boyonova *et al.* (2006) reviewed 118 case records. In that study anaerobes were prevalent in 71% of cases, with *Prevotella* (49%), a gram-negative bacilli, again being the most common anaerobe. *Fusobacterium* (22%), also a gram-negative organism, was found to be the second most common anaerobe.



2.3 Pus sampling:

The ideal transport system is essential in bacterial survival of bacteria. Smyth *et al.* (1993) conducted a study on pus swab sampling and time interval until specimens were processed by the microbiology laboratory. They confirmed the superiority of a trans swab over a non-trans swab for culturing organisms involved in cervico-facial infections. A trans swab contains Amies solution that keeps organisms viable for longer periods. The study also indicated that successful growth of some organisms was possible even after 72 hours with a trans swab, although clinically important anaerobic organisms such as *Peptostreptococcus* were no longer viable after 24 hours.



Tygerberg Academic Hospital and the Tygerberg Oral Health Centre contract the National Health Laboratory Services (NHLS) for the processing of microbiological specimens. The NHLS Microbiology Specimen Sampling Manual states that Syringe aspirates (three to five millilitres) are preferred over pus swab samples. In addition, if a delay of more than 30 minutes is anticipated until pus sample incubation, then the specimen should be transferred inside an anaerobic transport container. The manual also states that if a pus sample cannot be obtained by aspiration, then a pus swab may be used, the swab must then immediately be placed into a suitable transport medium (e.g. Amies solution/trans swab). Dry swabs are noted to be unacceptable.



Figure 1: Pus swab Amies medium

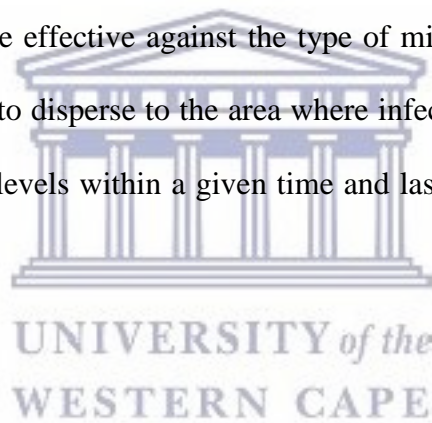
2.4 Antimicrobial therapy:

2.4.1 Background

There is a clinical significance for diagnosing gram-negative anaerobic organisms in cervico-facial sepsis (Gill and Scully 1988). Anaerobic organisms make up a substantial portion of human oral flora, and are subjected throughout life to antimicrobial drugs used in clinical therapy, which may result in a selection of resistant strains (Jenkins 2001). Antimicrobial resistance may complicate antimicrobial treatment of cervico-facial sepsis and influence therapeutic decisions of antimicrobial therapy in cervico-facial sepsis (Stefanopoulos 2004).

Rega *et al.* (2006) stated that empirical therapy should be initiated with penicillin and that metronidazole may be added. Other agents such as clindamycin and second or third generation cephalosporins may also be used. Empirical therapy is continued until microbiological sensitivities become available.

Holmes and Pellecchia (2016) mentioned that the common duration for antibiotic therapy in cervico-facial sepsis is seven to ten days. The authors also noted that host and pharmacological factors should be considered before prescribing antibiotics. Host factors include possible allergies of the patient, morbidities that the patient may have had with previous antibiotic therapies, pregnancy, and age as well as the immune status of the patient. A history of penicillin allergy can usually be elicited from the medical history. Long term use of, for example amoxicillin, will alert to a possible microbial resistance. Patients with impaired immune status will require specific bactericidal antibiotic regimens. Certain antibiotics may be contraindicated in the pregnant patient. Pharmacological factors include spectrum of the antibiotic, pharmacokinetics, cost as well as tissue distribution characteristics. The type of antibiotic prescribed should be effective against the type of microbial causing the infection. The antibiotic should be able to disperse to the area where infection is present and should be able to reach adequate blood levels within a given time and lastly, the medication should be affordable.



2.4.2. Antimicrobial agents

The following are all antimicrobial agents described in the literature for the treatment of cervico-facial sepsis:

2.4.2.1. Penicillin:

Heimdahl *et al.* (1980) refers to penicillin, a beta-lactam antimicrobial agent, as the original drug of choice in the treatment of cervico-facial sepsis. This family of beta-lactam antibacterials includes penicillin derivatives, cephalosporins, carbapenems and beta-lactamase inhibitors. These drugs are all active against gram-positive organisms as well as certain

anaerobes. Heindahl *et al.* 1980 however noted treatment failures in patients treated with penicillin for odontogenic cervico-facial sepsis. The researcher attributes these treatment failures to penicillin resistance.

A study by Lewis *et al.* (1995) examined 78 patients with suppurative oral infections. They found 23% resistance to penicillin. Their study indicated that gram-negative anaerobic bacilli are particularly prone to be penicillin resistant.

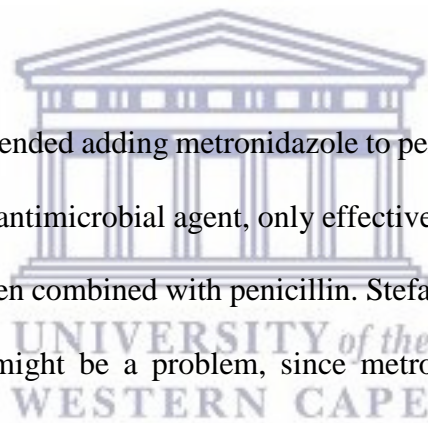
Penicillin is regarded as the drug with one of the most frequent medication allergies. Shenoy *et al.* (2019) reported that approximately 10% of patients were allergic to penicillin.

2.4.2.2. Metronidazole:

Stefanopoulos (2004) recommended adding metronidazole to penicillin as an add-on treatment for cervico-facial sepsis. This antimicrobial agent, only effective against strict anaerobes, adds additional anaerobic cover when combined with penicillin. Stefanopoulos (2004) further noted that medication compliance might be a problem, since metronidazole and penicillin have different dosing schedules.

2.4.2.3. Clindamycin:

Clindamycin prescription in cervico-facial sepsis is generally in patients allergic to penicillin (Stefanopoulos 2004). Johnson (1999) mentioned that clindamycin has a superior penetration into abscesses and 'jawbones' to that of penicillin. Reese (2000) noted an additional advantage over penicillin, in that clindamycin has a broader anaerobic spectrum. This allowed for clindamycin prescription as a monotherapy in cervico-facial sepsis.



In the past clinicians were reluctant to prescribe clindamycin due to the risk of superinfection with *Clostridium difficile* (Kuriyama 2001). Evidence in the scientific literature indicates that *Clostridium difficile* diarrhoea may occur with any broad-spectrum antibiotic (Hupp 2002).

2.4.2.4. Cephalosporins:

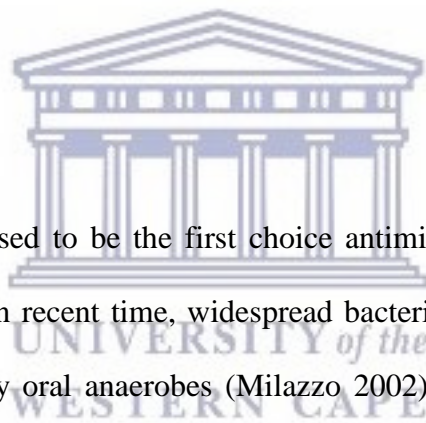
The cephalosporins, similar to penicillin, are beta-lactam antimicrobials. Cephalosporins are effective against aerobic and anaerobic gram-positive cocci. They are however less effective against gram-negative anaerobic bacilli (Flynn 2003). Cefoxitin is an exception to the rule as it demonstrates high levels of activity against gram-negative anaerobes (Brazier 2003).

2.4.2.5. Erythromycin:

Erythromycin, a macrolide, used to be the first choice antimicrobial in patients allergic to penicillin (Guralnick 1984). In recent time, widespread bacterial resistance to erythromycin has developed from especially oral anaerobes (Milazzo 2002). The use of erythromycin is mostly indicated for mild odontogenic infections (Petersen 2001).

2.4.2.6. Fluoroquinolones:

The fluoroquinolones group has an extended gram-positive and anaerobic spectrum (Flynn 2003). Fluoroquinolones such as gatifloxacin and moxifloxacin, show an acceptable activity against a wide spectrum of oral anaerobes (Sobottka 2002).



2.4.2.7. Aminoglycosides:

Aminoglycosides includes antibiotics such as gentamycin, streptomycin, amikacin and tobramycin. These antibiotics are especially active against gram-negative aerobes, which includes *Pseudomonas aeruginosa*. They have little activity against anaerobic organisms (Brook 2007).

2.4.2.8. Glycopeptides:

Clinically, the most relevant glycopeptides available are vancomycin and teicoplanin. Their spectrum is limited to gram-positive bacteria that includes methicillin resistant *Staphylococcus aureus*. Glycopeptide use is restricted to life threatening gram-positive infections. Nephrotoxicity is a particular concern with glycopeptides (Biavasco *et al.* 2000).



2.5 Cervico-facial sepsis

2.5.1. Introduction

Cervico-facial sepsis can occur from several sources i.e. odontogenic, cutaneous or salivary (Farmahan *et al.* 2014). They further stated that odontogenic infection accounted for 74% of infections with cutaneous infection accounting for 11% of cervico-facial sepsis.

Huang *et al.* (2006) grouped cervico-facial infectious sources into three groups: odontogenic, upper airway and other. The upper airway group includes sinusitis, peritonsillar abscess, supraglottic laryngitis and tonsillitis. The other sources group consisted of penetrating traumatic

injuries, periodontal and salivary. Their study indicated that odontogenic infection comprised 22% of cervico-facial infections, upper airway 18% and other sources 62%.

Huang *et al.* (2006) and Farmahan *et al.* (2014) both stated that infections involving the head and neck were polymicrobial in nature. They further stated that aerobic as well as anaerobic organisms were consistently present irrespective of the source of infection. Huang *et al.* (2006) demonstrated anaerobes present in three percent of cases where there was an odontogenic source, seven percent of cases with an upper airway source and five percent of infections from other sources. The researcher also reported that the majority of anaerobes stained gram-negative.



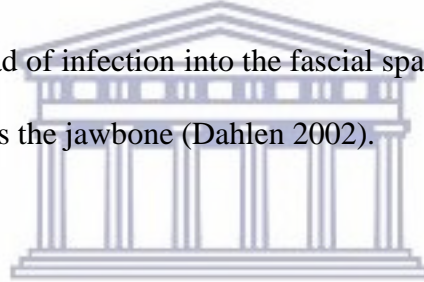
Figure 2: Cervico-facial sepsis complicated by gram-negative infection

2.5.2. Odontogenic infections Odontogenic infections:

According to Dahlen (2002), odontogenic infections consist of three main types: periapical abscesses, periodontal abscesses and pericoronitis.

2.5.2.1 Periapical abscess:

Periapical abscesses results from irreversible pulpitis and is associated with pulpal necrosis. The dental pulp is in direct connection with the periodontal ligament allowing bacteria to spread directly to the apical periodontal area. Formation of an abscess at the dental root apex can then result in further spread of infection into the fascial spaces of the head and neck. This occurs if the abscess penetrates the jawbone (Dahlen 2002).



2.5.2.2 Periodontal disease:

Periodontal disease is an infection of the bone and soft tissue that support teeth. The disease is divided into gingivitis and periodontitis. Gingivitis is inflammation restricted to the gingiva without alveolar bone involvement. Periodontitis is associated with alveolar bone loss and the formation of periodontal pockets. A complication of periodontal disease is the formation of a periodontal abscess. Anaerobic organisms are frequently seen in spreading periodontal disease (Dahlen 2002).

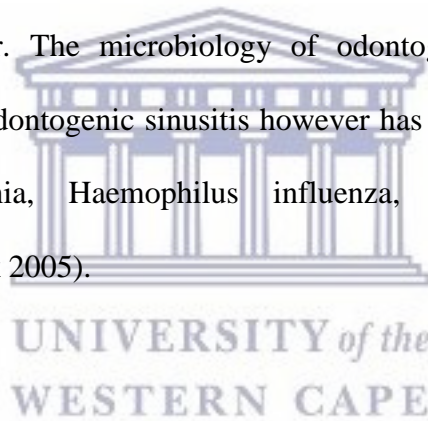
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2.5.2.3. Pericoronitis:

Pericoronitis is an infection of the gingiva surrounding the crown of a partially erupted tooth. The mandibular third molar is the most common cause of pericoronitis. The condition can become a serious since infection may spread rapidly. The microbes involved are poly-microbial with aerobes and anaerobes present (Dahlen 2002).

2.5.3. Sinusitis:

Suppurative sinusitis may be classified into odontogenic and non-odontogenic. Odontogenic sinusitis is mostly from carious maxillary molars or premolars that lie in close approximation to the maxillary sinus floor. The microbiology of odontogenic sinusitis is similar to odontogenic infection. Non-odontogenic sinusitis however has more aerobic organisms such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* (Brook 2005).



2.5.4. Peritonsillar abscess

Peritonsillar abscess is an abscess of the space between the tonsil and pharynx. It usually occurs as a complication of tonsillitis. An abscess that penetrates the superior pharyngeal constrictors will result in lateral pharyngeal space involvement. The infection may then spread further resulting in upper airway obstruction. The infection consists of both aerobic and anaerobic organisms (Brook 2007).

2.5.5 Suppurative arthritis of the Temporo-Mandibular Joint:

Suppurative infection involving the Temporo-Mandibular Joint (TMJ) is rare. The infection can be acute or chronic. Acute infections have the potential to spread to the temporal space. Significant destruction of the TMJ may also occur leading to joint ankylosis. Suppurative arthritis may occur from penetrating injuries, adjacent osteomyelitis of the jaws or from haematogenous bacterial dissemination. Streptococci and Staphylococci are frequently isolated (Sembronio 2007).

2.5.6 Actinomycosis:

The most common site for actinomycosis is in the maxillo-facial region (Miller 1998). The condition is characterised by an indurated swelling of the soft tissue with multiple abscesses. Later the infected area may develop multiple fistulae on the skin (Miller 1998). The infection is associated with poor dental hygiene, intra-oral trauma, dental infection, chronic mastoiditis and tonsillitis. The infection is usually polymicrobial. Gram-negative bacilli usually also form part of the infection.

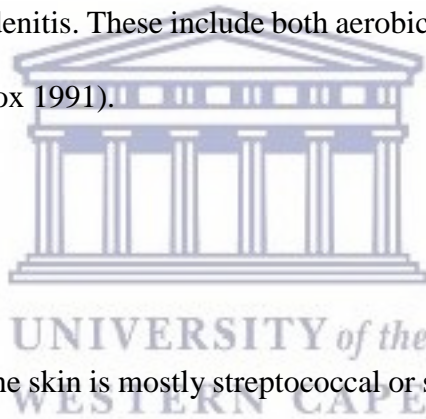
2.5.7 Tuberculosis:

Cervico-facial tuberculosis usually manifests as secondary lesions of pulmonary tuberculosis. Intra-orally, tuberculosis typically presents as irregular ulcers, frequently on the dorsum of the tongue. The skin of the head and neck may also be affected. The skin usually develops a painful red nodular appearance (lupus vulgaris). Tuberculosis can cause a cervical lymphadenitis. The involved nodes may later suppurate and form chronic draining sinuses (Murray 2007).

Salivary tuberculosis presents as a slowly enlarging mass in the area of a salivary gland. Diagnosis usually requires tissue from fine needle aspiration or core biopsy (Suoglo 1998).

2.5.8 Sialadenitis:

Infection of the salivary glands may be viral or bacterial. A variety of viruses can affect the salivary glands. Mumps caused by a paramyxovirus is the most common cause of viral sialadenitis. Other causes of viral sialadenitis are Coxsackie virus, Epstein-Barr and para-influenza B. Bacterial sialadenitis occurs mostly in patients where salivary calculi are present. Parotitis may occur in post-surgical patients secondary to dehydration. A variety of organisms is implicated in bacterial sialadenitis. These include both aerobic and anaerobic gram-positives and gram-negative bacteria (Fox 1991).



2.5.9 Skin infection:

Bacterial infection involving the skin is mostly streptococcal or staphylococcal. Erysipelas is a cellulitis of the skin secondary to streptococcal infection. The organism gains entry through the skin through small abrasions.

Staphylococcal infection of the superficial hair follicle is known as folliculitis. Deep infection of the hair follicle is called furunculosis (boil). Infection of adjacent hair follicles is a carbuncle. Impetigo is a superficial staphylococcal skin infection that forms an exudative area with crusting on the face. A severe bacterial infection of the skin is necrotizing fasciitis. This condition is associated with extensive necrosis of the skin. The infection may be polymicrobial or may only consist of staphylococci and streptococci.

Common, viral infections involving the head and neck include herpes zoster (shingles), herpes simplex and human papilloma virus. Herpes simplex is divided into primary and recurrent herpes simplex. Primary herpes simplex occurs mostly in children where the condition may be asymptomatic or present with primary herpetic gingivostomatitis, which presents with painful erosions of the oral mucosa and lips. Recurrent herpes presents as an ulcer of the lower lip usually. Shingles is a reactivation of chickenpox. The condition affects a dermatome. In the head and neck, the trigeminal nerve is mostly involved (Graham-Brown 2007).

2.6 Gram-negative anaerobes:

A study by Finegold *et al.* (1993) highlighted the important role that gram-negative anaerobes have in human infection. They noted that gram-negative organisms were mostly part of a polymicrobial infection, and that a substantial number of gram-negative organisms produced virulence factors that played a role in bacterial synergism. They also reported that a number of gram-negative anaerobes were noted to produce beta-lactamase and may be resistant to beta-lactam antibiotics. The researchers brought to attention the large amount of gram-negative infections that occurred in humans (Table 1).

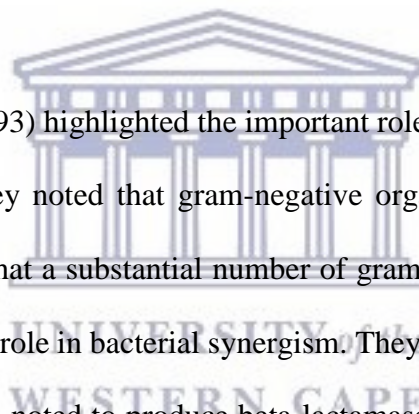


Table 1: Infections caused by gram-negative anaerobes (Finegold 1993)

<u>Central nervous system</u>	<u>Head and neck</u>	<u>Intra-abdominal or retroperitoneal</u>
Brain abscess	Membranous tonsillitis	Peritonitis
Subdural empyema	Peritonsillar abscess	Intra-abdominal abscess
Spinal epidural abscess	Retropharyngeal abscess	Perforated peptic ulcer
Septic cortical thrombophlebitis	Ludwig's angina	Appendicitis
	Phlegmon floor of mouth	Diverticulitis
	Lateral pharyngeal space infection	Subphrenic abscess
	Peri-mandibular space infection	Liver abscess
	Chronic sinusitis	Psoas abscess
	Chronic otitis media	
	Mastoiditis	<u>Bone and joint</u>
	Parotitis, parotid abscess	Osteomyelitis
	Sub mandibular gland abscess	
		<u>Skin</u>
		Human and animal bites
		Burn wounds
		Paronychia
<u>Dental</u>		
Endodontal infection		
Periodontal disease		
Juvenile periodontitis		
Pyogenic granuloma		
Odontogenic infection		
<u>Genital tract</u>		
Pelvic inflammatory disease		

Chapter 3

AIM AND OBJECTIVES

3.1. Aim

The aim of the research was to assess the prevalence of gram-negative staining bacteria in a population of patients with cervico-facial sepsis and to determine the number of gram-negative stains that yielded gram-negative organisms on culture. Factors influencing microbiological laboratory results were also considered.

3.2. Objectives

The objectives of the study were:

- 1) to determine the prevalence of gram-negative bacteria on microscopy and culture
- 2) to establish the number of gram-negative bacterial cultures obtained from those pus swab samples that stained gram-negative
- 3) to analyse the cultures obtained from the trans- and non-trans swabs
- 4) to identify and analyze other factors that may influence the results of gram staining and culture.

3.3. Research hypothesis

Pus swab specimens with a gram-negative stain do culture gram-negative organisms when using pus swabs as collection medium.

Chapter 4

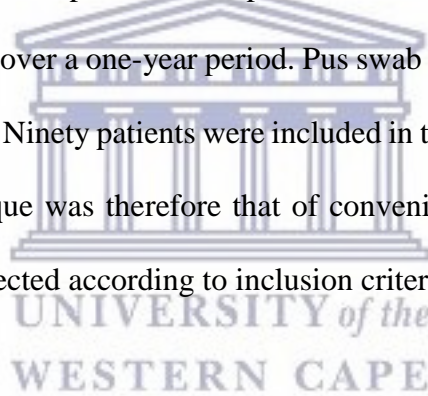
MATERIALS AND METHODS

4.1. Study design

The study design was a retrospective analytical cross sectional study.

4.2. Sample Size

The population group consisted of patients with pus swabs taken for cervico-facial sepsis at Tygerberg Oral Health Centre over a one-year period. Pus swab results were collected from 01 May 2017 until 30 April 2018. Ninety patients were included in the study that met the inclusion criteria. The sampling technique was therefore that of convenience sampling, where patient records were sequentially collected according to inclusion criteria over a one-year period.



4.2.1 Inclusion and exclusion criteria

Inclusion criteria were:

- 1) Pus swab results of patients that had incision and drainage performed at the Tygerberg Oral Health Centre for cervico-facial infection from 01 May 2017 to 30 April 2018.
- 2) Report that included gram stain and culture results.
- 3) Report that included recorded collection and laboratory registration time.

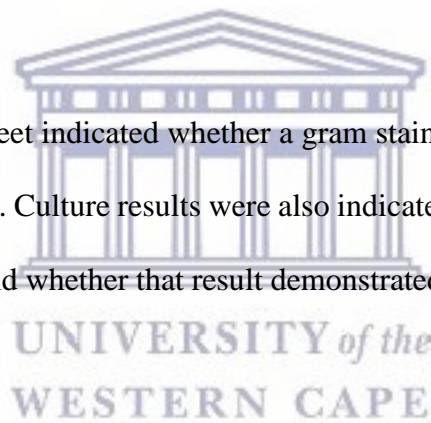
Exclusion criteria were:

- 1) Pus swab results with no gram stain or culture result reported.
- 2) Pus swab results without a collection and laboratory registration time.
- 3) Results of patient that fell outside the period of 01 May 2017 to 30 April 2018.

4.3. Data collection and analysis

The quantitative data (pus swab results) collection was through the NHLS database, using a database search that identified patients that had pus swabs taken for cervico-facial sepsis over a one-year period. These results were then tabulated in Microsoft® Excel.

The Microsoft Excel spreadsheet indicated whether a gram stain result was present and if that stain result was gram-negative. Culture results were also indicated on the spreadsheet noting if a culture result was present and whether that result demonstrated gram-negative organisms on culture.



Other variables, which could influence the culture and gram stain results were considered i.e. whether two different pus swabs were used during the pus sampling, and whether certain pus swabs may have registered after 24 hours.

A statistics software program (GraphPad Prism 8®) was used to calculate if trans swabs compared to non-trans swabs were statistically more likely to culture gram-negative organisms and also to calculate if pus swabs registered within 24 hours of being obtained, were statistically more likely to have a culture result.

4.4. Ethical consideration

This was a retrospective study of patient results obtained from the NHLS records. Permission was obtained from the head of the NHLS. All patient information included in the study was stored numerically and sequentially to maintain patient anonymity. Patient records were stored on a password-protected computer and printed information was stored in a locked office.

This mini-thesis proposal was presented to the Faculty of Dentistry's Research Committee at the University of the Western Cape and was approved by the Biomedical Research Ethics Committee (approval number: BM/18/6/6), University of the Western Cape (Appendix II).



Chapter 5

RESULTS

Ninety pus swab specimens met the criteria for inclusion, of which 71 pus swab specimens stained reactive for the presence of microorganisms. Of the 71 pus swab specimens that stained reactive for the presence of microorganisms, 48 (53%) pus swab specimens stained gram-negative. Of the 48 gram-negative staining pus swab specimens, only two gram-negative organisms (4%) were cultured (Figure 3).

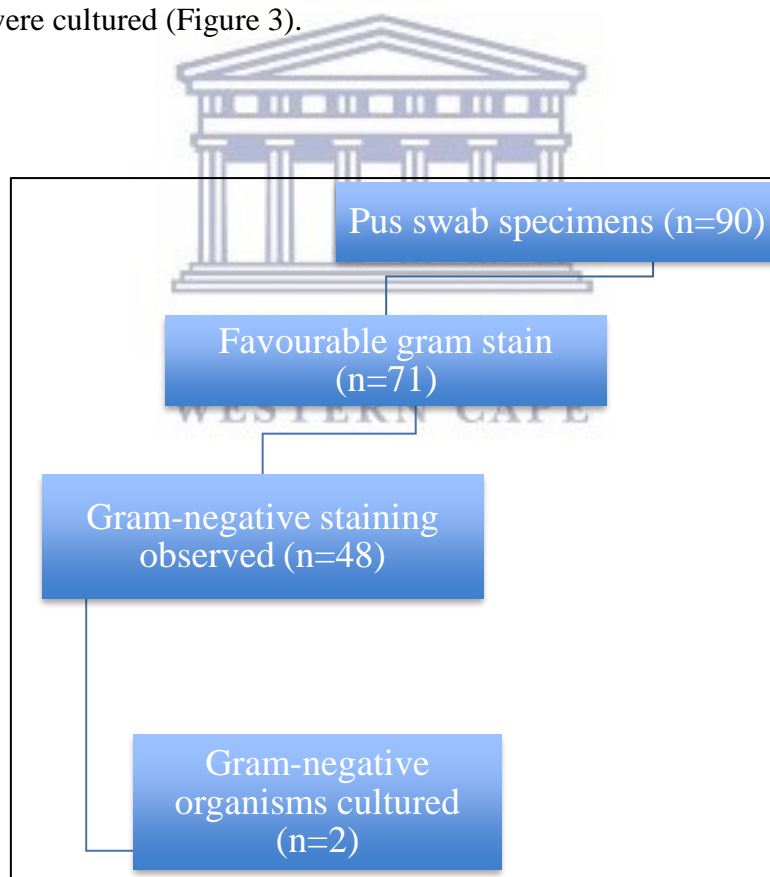


Figure 3: Results of gram stain

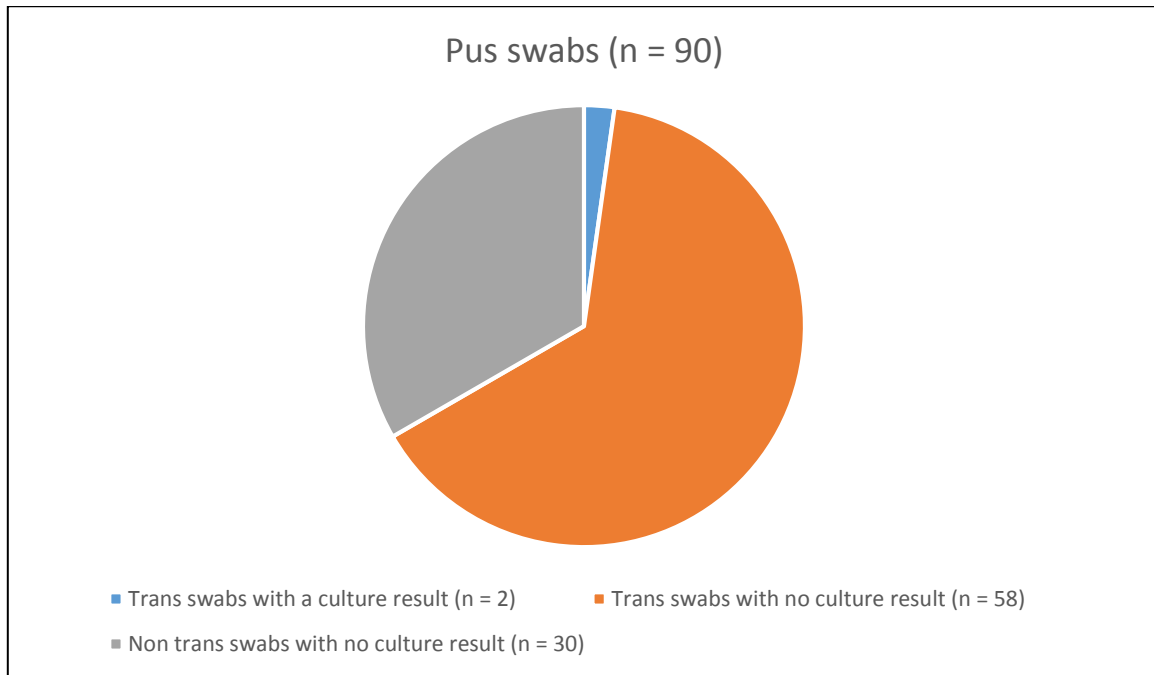


Figure 4: Trans vs. non-trans swabs

Ninety pus swabs were used of which 60 were trans and 30 were non trans swabs (Figure 4). The two gram-negative organisms cultured were taken with a trans swab. A Fisher Exact test comparing trans swab to non-trans swab, indicated no statistically significant difference ($p = 1$) and that trans swabs and non-trans swabs were statistically equally likely to culture gram-negative organisms.

Of the 71 pus swab samples staining favourable for the presence of microorganisms, 14 (19%) pus swab specimens were registered after 24 hours and 57 (81%) specimens were registered within 24 hours. The 14 pus swab specimens registered after 24 hours had six cultures present and the 57 pus swab specimens registered within 24 hours had 48 cultures present (Figure 5).

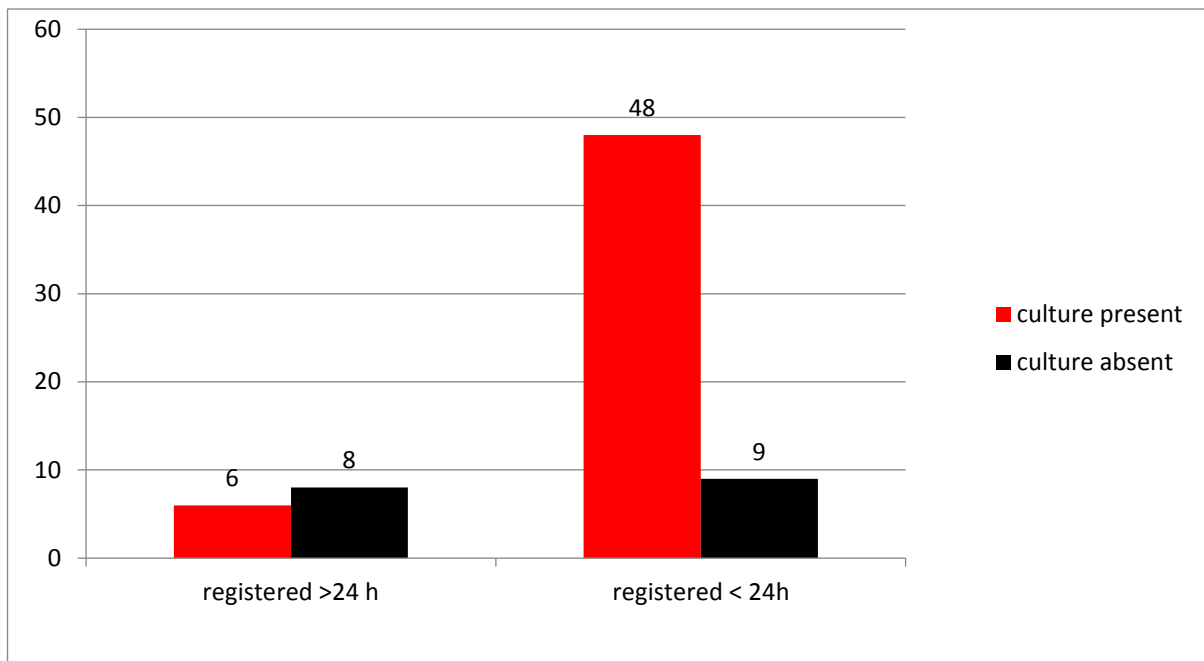


Figure 5: Presence or absence of bacterial culture for different time intervals (h=hours).

Chi square test (chi-square value = 10,5) indicated that pus swabs registered within 24 hours has a statistically higher probability of culturing organisms ($p = 0,0012$).

Of the 71 pus swab specimens that stained favourable on gram stain, 81% ($n = 57$) were registered within 24 hours. From that group, only two gram-negative organisms were cultured (Table 2).

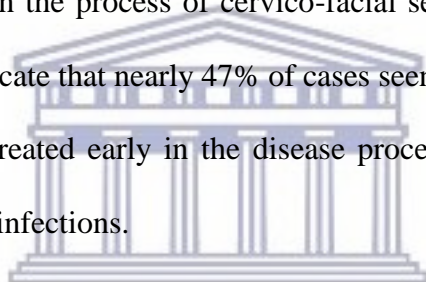
Table 2: MCS result of the two positive gram-negative cultures

Bacterial Culture	Antibiotic/culture	ENTCC
1. Enterobacter Cloacae Subsp Cloacae	Ampicillin / Amoxicillin Amoxicillin-clavulanic acid Ciprofloxacin Cefuroxime (Parenteral) Cefuroxime (Oral) Cefepime Gentamicin Piperacillin/	R R S R R S S S S
2. Enterobacter Cloacae Subsp Cloacae	Ampicillin / Amoxicillin Amoxicillin-clavulanic acid Ciprofloxacin Cefuroxime (Parenteral) Cefuroxime (Oral) Cefepime Gentamicin Piperacillin/	R R S R R S S S S

Chapter 6

DISCUSSION

In this study, gram-negative staining organisms were present in 53 percent of pus swab specimens that were processed for cervico-facial sepsis. This is comparable to the literature where Boyanova *et al*, (2006) found an incidence of 51.7% gram-negative organisms in a study involving 90 cases of head and neck infections. The literature indicated that gram-negative organisms predominate later in the process of cervico-facial sepsis (Rega *et al*. 2006). This result could therefore also indicate that nearly 47% of cases seen at the Tygerberg Oral Health Centre (TBOHC) were thus treated early in the disease process, before they progressed to predominantly gram-negative infections.



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Of concern to the researcher was that only four percent of the gram-negative pus swab specimens cultured gram-negative organisms. The TBOHC sends all pus swabs to the National Health Laboratory Services (NHLS). The NHLS guidelines recommend using an anaerobic transport vial when taking pus samples to culture gram-negative organisms, since most gram-negative organisms are predominantly anaerobes. The NHLS guidelines also recommend submitting at least three millilitres of pus for gram staining and culture.

In this study, pus sampling was performed with a pus swab. These swabs would have sampled less than three millilitres of pus and would not have kept organisms in an anaerobic environment. Non-adherence to NHLS laboratory pus sampling guidelines meant that none of

the 48 samples (53%) that confirmed the presence of gram-negative organisms on staining would have been cultured under optimal anaerobic conditions.

The two transport mediums used were analysed as possible factors, and there was found to be no statistical significance between the two mediums in regard to gram-negative culture propensity. It must be noted that many of the gram-negative organisms seen in cervico-facial sepsis are anaerobes. The swabs used in the study were found to be suboptimal for culturing gram-negative organisms.

The literature indicates that time delays until registration of pus swab specimens by the laboratory could affect the culturing process. Pus swab specimens registered within 24 hours of sampling have a higher probability of culturing organisms (Smyth *et al.* 1993). The majority (81%) of pus swab specimens in this study was registered within 24 hours.

The solution to improved identification of anaerobic organisms in cervico-facial sepsis would therefore be to increase awareness to NHLS pus sampling guidelines. Measures that may help improve awareness of NHLS pus sampling guidelines may include displaying the NHLS pus sampling guidelines in Maxillo-Facial and Oral Surgery clinics or even incorporating the guidelines into the undergraduate and postgraduate curricula.

It was noted that both the gram-negative cultures were resistant to penicillin and sensitive to the aminoglycosides. The question can therefore be asked whether gram-negative antimicrobial therapy might not play a more substantial role in cervico-facial sepsis if optimal pus sampling is implemented.

Managing cervico-facial sepsis ineffectively has implications regarding unnecessary treatment cost, inaccurate antibiotic prescription and increased patient morbidity.



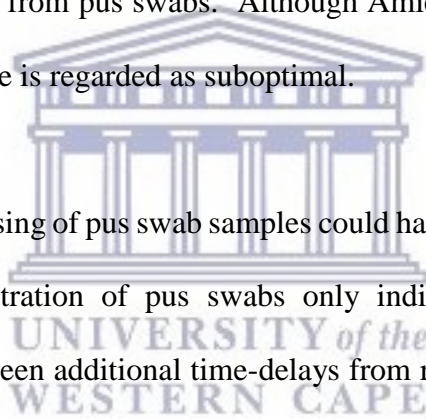
Chapter 7

LIMITATIONS

There were a few limitations in the study. Certain patients, unknown to the researcher, may have received antibiotics prescribed by the referring clinician prior to presenting at the TBOH for incision and drainage. This meant that certain pus specimens might be unrepresentative of the original infectious organisms, due the effect of previous antibiotic treatment.

All specimens were processed from pus swabs. Although Amies Elution swabs are accepted for anaerobic cultures, their use is regarded as suboptimal.

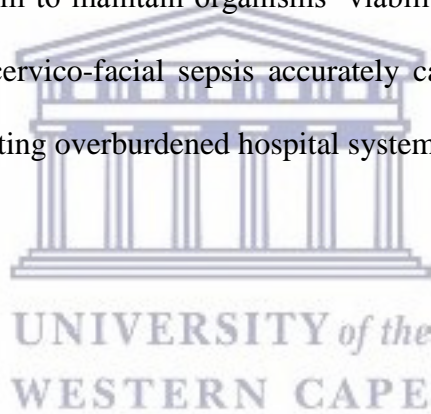
Time-delay until actual processing of pus swab samples could have also influenced the findings as the time-delay until registration of pus swabs only indicated when specimens were registered. There could have been additional time-delays from registration of specimens until actual specimen processing. These additional time-delays would have exposed anaerobic organisms to an aerobic environment for additional periods.



Chapter 8

CONCLUSION

It is clear that cervico-facial sepsis microbiological diagnoses during the period under review was sub-optimal. Gram-negative organisms of which the majority are anaerobes were not being cultured from pus swabs taken for cervico-facial sepsis. It is therefore essential that clinicians follow the microbiology laboratory protocols when microscopy, culture and sensitivity are requested. One of the crucial factors affecting the possibility of detecting pathogens is the efficacy of the transport system to maintain organisms' viability until the clinical sample is fully processed. Managing cervico-facial sepsis accurately can have a positive impact in relieving the strain on the existing overburdened hospital system in South Africa.

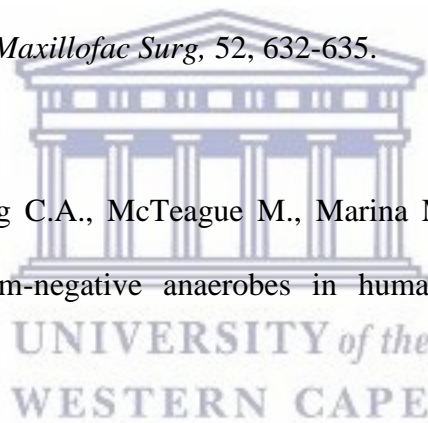


Chapter 9

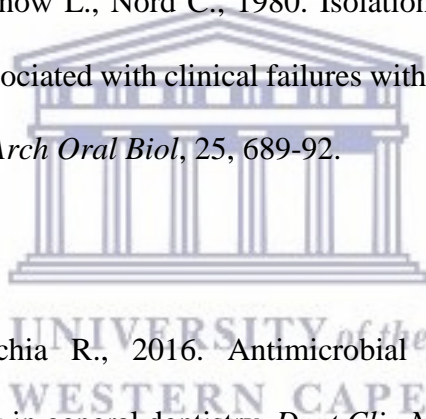
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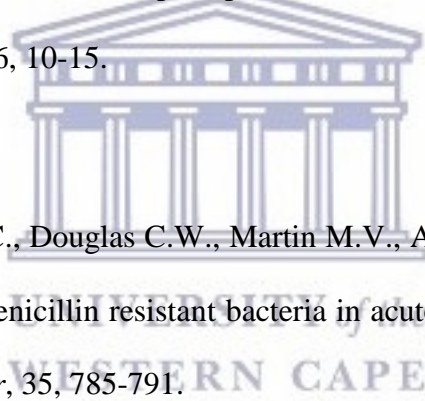
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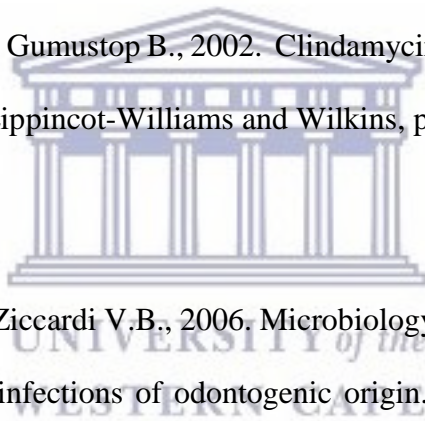
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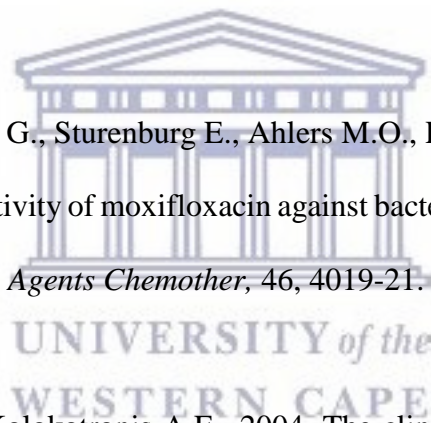
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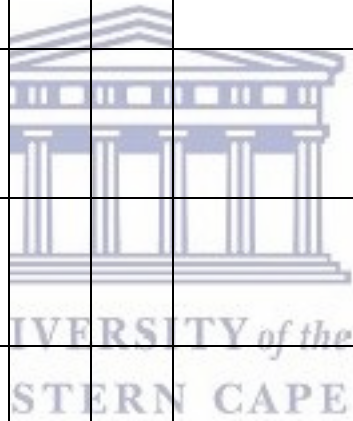
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Appendix I: Example of data capturing form

Case/swab no	Gram stain	gram positive 1	gram negative 2	both positive and negative 3	no organisms on gram stain 4	organism on gram stain 5	culture result	no organisms on culture 6	organisms on culture 7	gram positive organism on culture 8	gram negative organism on culture 9	>24h 10



Appendix II: Ethical clearance



OFFICE OF THE DIRECTOR: RESEARCH
RESEARCH AND INNOVATION DIVISION

Private Bag X17, Bellville 7535
South Africa
T: +27 21 959 4111/2948
F: +27 21 959 3170
E: research-ethics@uwc.ac.za
www.uwc.ac.za

21 September 2018

Dr N Barnard
Faculty of Dentistry

Ethics Reference Number: BM18/6/6

Project Title: Prevalence of frame-negative infections in cervicofacial sepsis.

Approval Period: 03 August 2018 – 03 August 2019

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report in good time for annual renewal.

The Committee must be informed of any serious adverse event and/or termination of the study.

A handwritten signature in blue ink, appearing to read 'Josias', is written over a faint watermark of a sunflower.

*Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape*

PROVISIONAL REC NUMBER -130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE.