OROFACIAL SEPSIS AND HIV AT MAXILLO-FACIAL SURGERY UNITS IN THE WESTERN CAPE

A PROSPESCTIVE STUDY

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A PROSPESCTIVE STUDY

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A Mini-thesis submitted in partial fulfillment of the requirements for the degree Magister Chirugiae Dentium in the discipline of Maxillo-Facial and Oral Surgery in the Faculty of Dentistry of the Western Cape.

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DECLARATION

I, Imraan Sarvan declare that '**OROFACIAL SEPSIS AND HIV AT MAXILLO-FACIAL SURGERY UNITS IN THE WESTERN CAPE: A PROSPESCTIVE STUDY'** is my own work and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

Signed: …….....

IMRAAN SARVAN

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DEDICATION

TO SIHAAM, MY LOVING WIFE AND BEST FRIEND

… My destiny, my significance

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ABSTRACT

The World Health Organisation estimated that in 2002 more than 13,772 000 deaths in developing countries were caused by infections. This accounted for more than 45% of all deaths, making up 7 of the top 10 causes of death (World Health Organisation, 2004). Sub-Saharan Africa is the epicentre of the devastating HIV pandemic. The country leading with the highest HIV rate in the world is South Africa, with approximately 5.5 million people infected (UNAIDS, 2008; South Africa Country Progress Report, 2008).

The development from HIV to AIDS progressively weakens the immune system, making the individual more susceptible to numerous infections, e.g. various forms of orofacial sepsis (Mindel, and Tenant-Flowers, 2001). HIV-positive individuals are eighteen times more likely to become infected with community-acquired methicillin-resistant *Staphylococcus aureus* than the general population. (Crum-Cianflone et al., 2006). The management of sepsis is of great concern with regard to human morbidity and mortality, as well as its financial implications, especially in cases of antibiotic resistance (Kimleck et al., 1976; Panlilo et al., 1992; Kirkland et al., 1999). Currently, there is no published peer-reviewed literature assessing the impact of HIV on orofacial sepsis.

This study aimed to assess the impact of HIV on orofacial sepsis, investigating the clinical and microbiological profiles of the population. These results were used as a guide in the adaptation of current treatment protocols.

The study population consisted of patients with orofacial sepsis (requiring incision and drainage or admission) who were referred to the Maxillo-Facial

and Oral Surgery Units at either Groote Schuur or Tygerberg Hospitals. These patients were examined, diagnosed and treated as per standard protocol. The empiric antibiotic treatment was tailored according to microscopy and sensitivity results when it became available. The exclusion criteria of the study were refusal of HIV testing or unwillingness of patients to participate in the study.

The **ratio of HIV positive** patients treated was much **higher than the population prevalence** (2.4:1.1). Odontogenic infections (71.11%), followed by septic jaw fractures (15.56%) were the most common causes of sepsis. The **most common causative teeth** were the mandibular posterior teeth (43.75%) (excluding the mandibular 3rd molars). The most common fascial spaces involved in the HIV positive group were the submandibular spaces (36%), followed by the submasseteric and canine spaces (27% each). In the HIV negative group, the buccal (41%) and submandibular spaces (33%) were the most common fascial spaces infected.

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The **HIV negative group** had the most **multi-fascial space** involvement, with **35%** having **more than one fascial space** involved. In comparison, the HIV positive group had only 18% involvement of more than one fascial space. This was also reflected by the **HIV negative group, which included five cases of Ludwig's Angina** as compared to one case in the HIV positive group.

The **Gram Stain** showed a predominance of **Gram positive cocci** for both the HIV positive and negative groups. **Gram positive bacilli** were **significantly more prevalent** in the **HIV negative group** ($p = 0.0409$). Pre-treatment antibiotics were associated with sterile abscesses in 20% of the cases. **No growth on culture** occurred only in the **HIV negative** group (statistically significant with $p = 0.00488$).

A statistically **significant increased length of admission** was found for the cases with **penicillin-resistant bacteria** (Wicoxin Rank Sum Test p = 0.0072). **Penicillin resistance** was found in **17.78%** (8 cases) with ten strains of five types of bacteria (*S. aureus, K. pneumonia, Enterobacter, E. coli, Alpha-haemolytic Streptococcus*). Eight percent (5) of these cases were **also resistant to co-amoxiclav®**.

In the **HIV positive group** the following **trends** ($p>0.005$) were found:

- The average **platelet counts** of this group was 112.34 $x10^8$/L (**lower** than the HIV negative group);
- The **length of admission** for the HIV positive group was slightly **longer** by 0.25 days even though this group had fewer fascial spaces infected;
- A larger **number of bacteria with penicillin-resistance** was more prevalent in the HIV positive group (six resistant bacteria in four cases compared to four resistant bacteria in four cases).

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Greater numbers of orofacial infections were seen in HIV positive subjects relative to their population prevalence rates. Added to this, was the higher rate of antibiotic resistance and longer hospital admissions. These findings may warrant further investigation of the relationship between HIV positive and negative groups with regard to orofacial sepsis.

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CHAPTER 1

INTRODUCTION

The World Health Organisation estimated that in 2002 more than 13,772 000 deaths in developing countries were caused by infections. This accounted for more than 45% of all deaths making up seven of the top ten causes of death. HIV–AIDS has been of the largest contributors, with an estimated mortality of 2,678 000 (World Health Organisation, 2004).

Sub-Saharan Africa forms the epicentre of the HIV pandemic, making up 90% of all paediatric cases and 68% of all the adult cases of HIV (UNAIDS, 2008). With South Africa having approximately 5.5 million people infected with HIV, it has the highest rate in the world (UNAIDS, 2008; South Africa Country Progress Report, 2008 Key HIV Statistics, 2008).

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The progression from HIV to AIDS may take up to 10 years or more. It progressively weakens the immune system making the individual more susceptible to numerous infections, e.g. various forms of orofacial sepsis (Mindel and Tenant - Flowers, 2001).

Management of infections with antibiotics has revolutionised medicine, starting with the discovery of penicillin by Nobel laureate, Alexander Flemming. Soon after his revolutionary break through, he discovered that this 'wonder drug' had become ineffective against certain *Staphylococcus* strains. He then correctly predicted that antibiotic failure would result with imprudent usage. It is now an accepted fact that sub-therapeutic doses create a situation where highly resistant strains can be selected sequentially.

In recently published literature, it appeared that HIV positive individuals may have a greater risk of antibiotic resistance compared to their HIV negative controls (Cotton et al., 2008; Madhi et al, 2000; Krucke et al, 2008). In a prospective study by Cotton et al. (2008), nasopharyngeal swabs and blood cultures were done on 203 HIV positive children. The authors found that resistance to Trimethoprim Sulphamethoxazole (TMP-SMX) occurred in more than 80% of all pathogens except with *M. cattarrhalis*. Methicillin-resistant *S. aureus* was significantly associated with TMP-SMX treatment ($p = 0.002$). They also found that 50% of *Entrobacteriaceae* produced extended spectrum β-lactamases (ESBL), making them resistant to third generation cephalosporins, and 56% were resistant to gentamycin. MRSA (resistant to penicillin and oxacillin) occurred in 77% of S. aureus cases. It was concluded that HIV positive children were colonised with potential pathogens which were resistant to commonly used antibiotics, and that antibiotic prescribers should take cognisance of these resistance patterns.

Krucke et al. (2008) conducted a study on adult HIV-infected individuals who developed cutaneous abscesses, and found that the most common microorganism was S. aureus (84.6%). Methicillin-resistant *S. aureus* was found in 85.7% of cases, but these organisms were not resistant to TMP-SMX, rifampicin or vancomycin. In the 2006 publication by Crum-Cianflone, he found that HIV-positive individuals were 18 times more likely to become infected with community-acquired methicillin-resistant S. aureus than the general population.

The management of sepsis is of great concern with regard to human morbidity and mortality, as well as its financial implications, especially in cases of antibiotic resistance (Kirkland et al., 1999; Panlilo et al., 1992). Surveillance of antibiotic resistance against common causative organisms should be used to establish sensitivity profiles. The sensitivity profiles should be disease-based to inform the development of, or amendment to, standard treatment guidelines and essential drugs lists (Iruka et el., 2003).

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In a 3-year retrospective study (Amaidas, 1990) conducted at one of the Maxillo-Facial and Oral Surgery Referral Units in the Western Cape, it was found that cervicofacial sepsis made up 4% (587 patients) of admissions. In the author's analysis of a randomly selected group (60 patients), he found two cases of antibiotic resistance to the first line antibiotics (amoxycillin or penicillin G and metronidazole). In both cases the bacteria was sensitive to ampicillin. This result does not appear to reflect the current antibiotic resistance profile as seen at the same Maxillo-Facial and Oral Surgery Unit and at its sister unit 20km away. It also appeared to that HIV positive patients presented with more odontogenic abscesses, and appeared to have more extensive abscess formation than the HIV negative population. Could HIV be the cause of increased antibiotic resistance? This could not be substantiated nor negated, as there is a sparsity of peer-reviewed publications dealing with orofacial sepsis comparing the HIV positive and HIV negative populations and their antibiotic resistance profiles.

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CHAPTER 2

LITERATURE REVIEW

2.1 Types of Flora

2.1.1 Normal Flora

It is usually comprises of combinations of indigenous bacteria and yeasts specific to anatomical regions of the body. These play a protective role against pathogenic organisms. The composition, numbers, and the distribution is determined by the anatomic region, host's immunity, oxygen and humidity levels, surface of the body and competition with other microbes (Koneman et al, 1988). The flora may change, be replaced by a transient flora, may return to normal flora or be changed permanently.

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2.1.2 Oral Flora

The evolution of the oral flora begins at birth and is representative of the mother's vagina, comprising of Enterococci, Streptococci and microaerophillic organisms (Koneman et al, 1988). Aerobic microflora predominate until eruption of teeth occur when the environment harbours more anaerobic flora. Diet, smoking, medication, alcohol, radiation and diseases (processes like caries and periodontal disease) all affect microflora composition (Koneman et al, 1988). The normal oral flora comprises of a combination of bacteria, fungi, yeasts, protozoa, spirochetes and viruses (Figure 1).

Figure 1.
Some of the most common indigenous and colonising oral flora [Haug, 2003]

Aerobic and facultative isolates
Diphtheroids
Lactobacilli
Rothia dentocariosa
Streptococcus faecalis
Streptococcus milleri
Streptococcus mitis
Streptococcus mutans
Streptococcus salivarius
Streptococcus sanguis
Anaerobic isolates
Actinobacillus actinomycetemcomitans
Actinomyces israelii
Actinomyces naeslundii
Actinomyces odontolyticus
Actinomyces viscosus
Bacteroides asaccharolyticus
Bacteroides melaninogenicus
Bacteroides oralis
Bacteroides ochraceus
Bacterionema matruchotii
Branhamella catarrhalis
Capnocytophaga gingivalis
Capnocytophaga ochraceus
Capnocytophaga sputigena
Eikenella corrodens
Fusobacterium plauti
Fusobacterium nucleatum
Neisseria sicca
Peptostreptococcus
Veillonella alcalescens
Veillonella parvula

Spirochetes
Spirochaeta species
Christispira species
Treponema denticola
Treponema orale
Treponema macrodentium
Treponema vincentii
Borrelia species
Leptospira species
Yeasts
Aspergillus species
Candida albicans
Other Candida species
Geotrichum species
Hemispora species
Penicillium species
Scopulariopsis species
Viruses
Herpes simplex virus
Cytomegalovirus
Protozoa
Entamoeba gingivalis
Trichomonas tenax

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2.1.3 Cutaneous Flora

The newborn's flora is representative of the birth canal. It is replaced by a combination of aerobic and facultative anaerobic bacteria, yeasts and sometimes even animal-carried organisms (Schuster, 2002) (Figure 2).

Figure 2.
Some of the most common indigenous and colonising facial cutaneous flora [Haug, 2003]

Aerobic and facultative isolates
Acinetobacter species
Enterobacteriaceae
Corynebacterium species
Micrococcus species
Staphylococcus epidermidis
Staphylococcus species
Streptococcus viridans
Yeasts
Candida albicans
Malassezia furfur
Pityrosporum ovale
Pityrosporum orbiculare
Torulopsis glabrata
Animal
Demodex folliculorum

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2.1.4 Nasal and Paranasal Flora

The nasal or paranasal sinuses are usually sterile at birth and become colonised by the environment it is exposed to (Bamberger, 1991). Nasal secretions contain enzymes and immunoglobulins that inhibit the growth of organisms, and may be colonised differently over time according to changed immunity of the host (Bamberger, 1991) (Figure 3).

Figure 3.
Some of the most common indigenous and colonising nasal and paranasal sinus flora [Haug, 2003]

Aerobic and facultative isolates
Corynebacterium diptheria
Corynebacterium species
Haemophilus influenzae
Haemophilus parainfluenza
Neisseria species
Staphylococcus species
Streptococcus pneumoniae
Moraxella species
Micrococcus species
Neisseria meningitidis
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus viridans
Streptococcus pneumonia
Anaerobic isolates
Propionibacterium acnes

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2.2 Major Causes of Orofacial Sepsis

2.2.1 Odontogenic Causes

In the early 1960s, teeth were listed as the fifth or sixth leading causes of death recorded on the London Bills of Mortality (Clarke, 1999). At the beginning of the twentieth century, dental infections were associated with a mortality rate of 10 - 40% (Turner-Thomas, 1908).

Odontogenic bacterial infection is still reported as being the *most common cause* of orofacial sepsis. This was illustrated by Amaidas (1990) who showed the prevalence of 56.7% for odontogenic only causes and 21.7%, where the tooth was left in the jaw line of fracture.

In a 4-year prospective study by Flynn et al. (2006a), they found that 65% of odontogenic abscesses were caused by caries, 22% by pericoronitis and 22% by periodontal infections. Other studies also supported the finding that odontogenic infections are the most common cause of orofacial sepsis (Woods, 1978; Morey et al., 1984; Guralnick, 1984).

With the improvement of laboratory protocols, the isolation of organisms has improved (Siqueira, 2001). The cultures from acute dental abscesses caused only by obligate anaerobes are approximately 20%. Depending on collection procedures, it may vary between 6 to 63% (Brook et al., 1991; Goumas et al., 1997; Khemaleelakul et al., 2002). Most cultures consist mostly of mixed obligate anaerobes and facultative anaerobes (59% to 75%), with *obligate anaerobes* outnumbering (1.5 to 3:1) facultative anaerobes. The mean number of bacteria cultured from dentoalveolar abscesses is four with a range of 1 - 7.5 (Robertson and Smith, 2009).

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In the study of the causative bacteria in orofacial sepsis reviewed by Haug (2003), and the study of bacteria in non-ondontogenic sepsis reviewed by Robertson and Smith (2009), they agreed that the causative pathogens have not changed much. However, it is the reclassification and nomenclature changes, as well as improved sampling, better transport media, improved protocols and the standardisation of laboratories, which have led to more species being cultured and identified.

The most commonly found facultative bacteria in abscesses are *viridans Streptococci* and *anginosis Streptococci*. The viridans group is subdivided into *mutans, salivarus, sanguinis, mitis* and *oralis* species. The anginosus group was previously identified as part of *β-haemolytic Streptococci* or they were divided as *Streptococcus melleri* or *Streptococcus anginosus* (Facklam, 2002).

Staphylococcus has more recently been identified as a frequent coloniser of oral environments. In the past, it was not considered to play a major pathogenic role

(Smith et al., 2001). In the review article by Robertson and Smith (2009) it was found that *S. aureus* was identified in 0.7 to 15% of acute abscesses. However, in the publication by Mangundjaja and Hardjawinata, (1990) it was reported that *S. aureus* was found in 47% of cases. In their review (Robertson and Smith, 2009), they also found that *S. epidermidis* was identified in up to 4 to 65% of cases.

The most common anaerobes are fusobacteria and the pigment anaerobes, *porphyromonas* and *Prevotella* (Robertson and Smith, 2009). The analysis of the literature in this group is very difficult due to the taxonomic identification changes in this group. The Bacteriodes group is now subdivided into the sacchrolytic genus, *Prevotella*, and the asacchrolytic genus, *Porhyromonas*. A part of this group is still being identified as the *Bacteriodes*, namely *fragilis* and *forsythus*, with the latter being more commonly identified in dental abscesses.

2.2.2 Trauma Related Causes

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Amaidas (1990), found that septic jaw fractures were the second most common cause of sepsis (21.7%). This was not unexpected, as the most common cause of admission to units in the Western Cape is trauma.

With trauma, the skin and or mucosa protective barriers are breeched allowing bacteria into the subcutaneous tissues. This occurs either by exposure (fracture open to periodontal ligament space) or by direct inoculation (breech of skin due to contaminated foreign object or even directly due to human or animal bites). The location of the injury and mechanism of injury will often determine the flora that initiates the infections.

The most common organisms in human bites are (aerobes) Streptococci, especially *angionosus, Staphylococci*, (anaerobes) *Eikenella corrodeus*, *Prevotella* and *Fusobacterium* (Talan et al., 1999).

According to the review by Talan et al. (1999), they found that *Pasturella* was the most common bacteria in both dog and cat bites (more in cat bites) and that *anaerobes* made up approximately 50% in dog bites and 60% in cat bites, with *Fusobacterium nucleatum, Bacteroides tectum, Prevotella heporinolytica* and several *phorphymonas* species being isolated. Rabies is also of concern, especially with wild animals or infected pet bites.

With midfacial fractures, organisms causing sepsis originate commonly from the nasal or paranasal sinuses or from cutaneous flora (Figures 2 and 3). Contamination with foreign material or debris often results in gram negative anaerobic infections (Brennen et al., 1990). Contamination with soil can result in *Clostridium tetani* and *Actinomyces* infections. With contaminated fresh water, the most common causative pathogens are *E. coli* (sea water as well), *V. cholera* or *Aeromona hydrophilia*.

With burns, maceration, avulsion or myonecrosis, the most common organisms are mixed infections. These organisms are anaerobic in nature such as *Clostridium perfringes*, *Bacteroides and Prevotella together with Streptococci* (Skitarelic et al., 2003).

Nosocomial infection is a great concern and occurs more often in immune-compromised patients and systemically ill patients. This is due to the high incidence of *multi-drug resistant* organisms in these patients. Staphylococcus aureus, *Enterococci, Klebsiella* and *Mycobacterium tuberculosis* are the most common pathogenic, fastidious multi-drug resistant organisms identified, with *differing patterns in each hospital,* (organism types and drug resistance profiles) (Kirkland et al., 1999).

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2.2.3 Nasal and Paranasal (Non-trauma Related) Causes

Sinusitis is often divided into acute and chronic maxillary sinusitis, infective rhinitis, frontal and sphenoid sinusitis. This is due to the differences in management and causative organisms. Maxillary sinusitis is divided into acute community-acquired bacterial sinusitis (ACABS), chronic and nosocomial sinusitis (Winther et al., 1998).

The most common organisms causing ACABS are *S. pneumonia* and *H. influenza* (approximately 50% of cases). *Staphylococcus aureus*, haemolytic Streptococci, *M. catarhalis, Streptococcus* species and anaerobic bacteria are also identified.

The role of *viruses* in ACABS can be up to 40% but is usually about 25% of cases (Gwaltney, 2000). According to Winther (1998), the organisms causing ACABS have remained unchanged except for their antibiotic resistance profiles. The resistance profiles are often geographic in nature with penicillin-resistant *S. aureus*, β–lactum resistant *M. catarrhalis, H. influenza*, as well as numerous strains of resistant *S. pneumonia* present. Often these strains form chronic sinusitis due to incorrect antibiotic management.

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Infectious rhinitis is classified into acute, self-limiting and chronic. The most common causes are *rhinoviruses, influenza* and *parainfluenza* viruses, creating a 'common cold' as part of the disease. Bacterial and fungal causes are not very common. In patients with weak *immune systems*, the following unusual pathogens can be found: *M. tuberculosis, aspergillosis, mucormycosis, syphilis, other fungi, protozoa and atypical mycobacteria* (Winther, 1998).

Frontal or sphenoid sinusitis is uncommon (< 5%) and have a *significant morbidity* associated. They are often caused by resistant strains of S. aureus, S. pneumonia and H. influenza. The frontal, sphenoid and chronic maxillary sinus infections are often surgically managed (Winther, 1998).

2.3 Cutaneous Infections

Cutaneous infections can be subdivided into bacterial, fungal, protozoal and viral causes. The most common bacterial cause is folliculitis, consisting of itchy, tender papules or pustules. This infection of the hair follicle may be superficial or deep, with infected follicle hair removed easily. The bearded area is normally involved (sycosis barbae) in subjects with stiff, curly beard hairs that re-enter the skin. Causative organisms are usually *S. aureus, C. albicans* and *P. aeruginosa*. Deeper seated, infected furuncles are usually caused by *Staphylococci*, occurring most frequently on the neck, breasts, face and buttocks. It begins as a nodule that becomes a pustule five to 30mm in size and develops central necrosis with discharge of necrotic core and purulent fluid. Small outbreaks may be common (furunculosis) in healthy individuals living together in poor hygiene conditions. Carbuncles are the more severe form and consist of clusters of furuncles with subcutaneous spread of pus, causing sloughing and slow healing scars. Carbuncles are often accompanied by systemic symptoms. All these conditions require antibiotic therapy, but folliculitis may also require topical fusidic acid application. *Surgical drainage* and sensitivity testing is also recommended for furunculosis and carbuncles (Kumar and Clarke, 1998).

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2.3.1 Erysipelas

Erysipelas is a condition that mimics cellulitis, primarily in young and elderly or diabetic patients. It is a superficial infection, which is commonly caused by β–haemolytic Streptococci Group A, and less often by the other Streptococci groups. Other bacteria associated are S. aureus, Pneumococcus, K. pneumonia, Y. enterlitica and H. influenza. The infection spreads via lymphatic channels giving the characteristic, fiery red face, which is *well circumscribed*, swollen, painful and indurated to touch. Penicillin is the mainstay of treatment. Complications from this condition vary from abscess formation, necrotising fasciitis, toxic shock syndrome, thrombophlebitis, acute glomerulonephritis, endocarditis, multi organ failure and

death. Recurrence of infections (erysipelas) is prone to damage lymphatics (Kumar and Clarke, 1998).

2.3.2 Tuberculosis

Tuberculosis in the orofacial region often manifests as a *lymphadenitis,* which may then breakdown with abscess and fistula formation (scrofula). It may also manifest as osteomyelitis with varying amounts of suppuration, often affecting the vertebrae or mandible. Virtually any system may be affected by *tuberculosis* including skin (lupus vulgaris), parotid gland, oesophagus, mucosa and middle ear. These forms of extrapulmonary tuberculosis are often associated with HIV-AIDS (50% will have extrapulmonary tuberculosis). Non-tuberculosis mycobacterial disease (*M. bovis*) from non-pasteurised, infected milk and *M. avium-intercellulare*, may often also cause lymphadenitis but can affect any organ system. Management is *drainage* of suppurative collections, *debridement* of necrotic bone (osteomyelitis) and *antituberculosis antibiotics* after microscopy and culture (Cleary and Batsakis, 1995).

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2.3.3 Gonorrhoea

Gonnorrhoeae is primarily a sexually transmitted disease but can affect the orofacial region after septicemic dissemination or directly via oral sex. The oropharynx demonstrates oedema and erythema with small punctuate pustules. It is caused by the sacchrolytic gram negative diplococci, N. gonorrhoeae. Treatment recommendation is the ‘syndromic’ approach for sexually transmitted diseases (Department of Health) due to the likelihood of multiple resistant pathogens. Partners should also be treated. The antibiotic combination would then cover all pathogens causing the same symptoms, e.g. N. gonorrhoeae and C. trachomatis. A single dose of either cefotaxime 1g intramuscularly; ceftriaxone 250 mg intramuscularly; ciprofloxacin 500mg orally; ofloxacin 400mg orally for 7 days; minocycline 100mg 12 hourly for 7 days or azithromycin 1g orally as a single dose, is advocated.

2.3.4 Necrotising Stomatitis (Cancrum Oris)

Necrotising stomatitis or noma, is a rapidly progressive infection in malnourished, immune compromised patients living in a poor hygiene environment. It is often preceded by other infections like *measles, tuberculosis, herpes simplex, varicella* and *bronchopneumonia*. Some authors believe it begins with necrotising ulcerative gingivitis (ANUG). It is caused by a mixed infection usually comprising of *F. necrophorum, F. nucleatum, P. intermedia, B. vincentii, S. aureus* and *non-haemolytic Streptococci* species. A related disorder, noma neonatorum, arises in low birth weight infants, usually in the first month of gestation. The organisms causing the paediatric form of disease, almost always consist of *P. aeruginosa*, often with *E. coli, Klebsiella* or *Staphylococcal* species (Falkler et al., 1999).

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2.3.5 Actinomycosis

Actinomycosis often affects the orofacial region (55%), 25% occurring in the abdominal and pelvic regions, 15% in the pulmonary system and 5% in the skin and genitourinary system. The infection may be acute, rapidly progressive or may be chronic in nature. It characteristically gives a suppurative infection with discharging yellow granules (called sulphur granules) which represent colonies of the gram positive anaerobic filamentous branching pathogen. It usually begins in an area of trauma or a previous extraction site. It then spreads typically, not following fascial planes, rather by direct extension and often forms numerous sinus tracts. Salivary gland and actinomycetic osteomyelitis is not unusual and often presents with very little pain. On microscopy, club–shaped filaments radiating in a rosette pattern with characteristic eosinohyllic periphery and basophyllic core (haematoxylin and eosin stain), can be seen. *Long-term penicillin* is the drug of choice (Miller and Haddad, 1998).

2.3.6 Cat–scratch disease

Cat–scratch disease presents as a papule or pustule at the site of a cat bite or scratch that forms three to 14 days after the injury. It usually heals by the time the lymphadenitis forms (draining node/s). Complications are suppuration, conjunctival granuloma (periorbital lesion), and temporary facial paralysis if the lymphoid tissues of the parotid gland is affected. Microscopy is required to identify the zonotic bacillary pathogen *Bartonella henselae*. The antibiotics of choice are *doxycycline and erythromycin* showing dramatic recovery in two days.

2.3.7 Acute Suppurative Lympadenitis

Acute suppurative lympadenitis most commonly occurs in children under four years old. This, according to Butler and Baker (1992), is due to the inability of the younger child's immune system to localise the organism at the site of attachment to the nasal or pharyngeal epithelium, allowing spread to nodes and resulting in suppuration. The most commonly affected nodes are the jugulodigastric and submandibular nodes. The most common causative organisms are S. aureus, S. pyogenes, with anaerobic organisms like *porphyromonas* and *Bacteroides* (Brook, 1987). Other bacteria like *tuberculosis* and *atypical tuberculosis* (*M. avium intracellulare*) also occur. According to Butler and Baker (1992) and Nuss and Cunningham (1993), HIV should also be regarded as an etiological agent. Management is incision and drainage if a collection is present and intravenous cloxacillin and metronidazole is recommended according to population resistance profiles.

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2.3.8 Necrotising Fasciitis

Necrotising fasciitis is a life threatening, rapidly spreading polymicrobial infection which causes oedema and necrosis of the fascia and subcutaneous tissues. The initiating cause of the infection is often minor trauma with contiguous spread of

infection like an abscess. This often occurs in individuals who are diabetic, alcoholic, immune suppressed (including HIV) and medically compromised. The most commonly affected regions are the extremities as well as the perineum, abdomen and head and neck regions. When the male genitalia are involved, it is called Fournier's disease. This disease begins with a very painful, red, hot and swollen skin and the patient displays signs and symptoms of systemic toxicity (high fever or hypothermia, tachycardia, hypotension, tachypnoea, hypoxemia, oliguria and often encountered, altered mental state). With progression, a volacious hue appears on the skin with the development of bullae or vesicles. Soon thereafter, the skin becomes anaesthetised with the development of frank necrosis. Microbiological analysis shows the occurrence of numerous species of *Group A β – haemolytic Streptococci*, as well as *Prevotella* or *Bacteroides*. In some cases *Clostridia* species can also be found.

Management requires intensive care admission with *broad spectrum antibiotics*, repeated *aggressive surgical debridement* (up to bleeding skin margins), antiseptic wound packing, *intensive medical support*, including fluid electrolytes and even blood products if required (Balcerak et al., 1988). *Hyperbaric oxygen* may also be of benefit to these patients (Langford et al., 1995).

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2.3.9 Herpes Simplex Infection

Herpes Simplex infection is considered as one of the most common infections of the orofacial region affecting mucocutaneuos regions of mostly the face and mouth, but can also affect the nasal and conjunctival structures. This lesion is rarely the cause of sepsis in the non-compromised host. HIV, organ transplant and malnourished individuals are susceptible to widespread and repeated infection associated with septic and possible orbital complications. Other complications that may occur in immune-compromised or susceptible groups, are herpetic witlow (infection of fingers), and herpes gladiatorum (infection of abraded skin lesions) (Neville et al., 2002).

2.3.10 Osteomyelitis

Osteomyelitis is an infectious process in malnourished or immune suppressed individuals causing an acute or chronic inflammatory process of the medullary and/or cortical surfaces of bone. There are several patterns identified other than the acute or chronic forms, namely focal and diffuse sclerosing osteomyelitis, proliferative periostitis and alveolar osteitis (Neville et al., 2002).

The most common precipitating causes are odontogenic infection and traumatic fracture of jaws. Other causes, often seen in developed countries, include acute necrotising gingivitis (ANUG) and cancrum oris (noma) (Adekeye and Cornah, 1985). The management of acute osteomyelitis is *intravenous antibiotics and drainage*. The antibiotics in acute osteomyelitis must cover odontogenic organisms. S. aureus and Salmonella often cause acute osteomyelitis in sickle cell disease. In cases of chronic osteomyelitis, S. aureus and Salmonella, with the addition of β – haemolytic Streptococcus and even sometimes actinomyces, are often identified as causative pathogens. (Flynn; Halpern, 2003).

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2.3.11 Uncommon Causes

Other causes, that are unlikely to form purulent infection, are cutaneous rhizopus, fungal infections (histoplasmosis, blastomycosis, candida, aspergilosis, and leptospirosis caused by Leptospira species) and Bubonic plague caused by Y. pestis.

2.4 Spread of Infection

As odontogenic infection has been shown to be the most common cause of abscess formation (Chow et al., 1978; Woods, 1978; Guralnick, 1984; Amaidas, 1990; Flynn et al, 2006a), the mechanisms and principles of spread of infection will be illustrated using odontogenic origins. The causes of odontogenic infection are most commonly

from carious teeth, pericoronitis, periodontitis, dentoalveolar or jaw fractures with teeth in the fracture line and exodontia (usually post surgical). Less common causes reported are loose implants (plates, screws and wires), septic grafts (jaw augmentation) and needle tract sepsis (post local anaesthetic).

According to Chavez de Paz (2007), after the pulp chamber is breached (carious tooth), a mixed bacterial colony is formed. This causes the wall of the root canal to become colonised by a special mixed anaerobic biofilm. Abscess formation occurs when bacteria and toxic products enter the periapical tissues via the apical foramen. Asymptomatic necrosis of the pulp may occur, but when periapical spread has occurred, symptoms manifest (pain, swelling, erythema).

After infection has occurred, it may remain localised or it may spread. The main routes of propagation are via haematogenous, lymphatic or by direct continuity (contiguous spread) (Shafer et al, 1983). Direct continuity is the propagation of the infection along anatomic pathways of least resistance (between fascia layers) (Bartlett and Gorbach, 1976).

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Roots of the teeth close to the buccal cortical plate generally spread purulent material buccally, while those close to the lingual or cortical plate, or maxillary sinus, generally spread to those closely associated structures. This may lead to the formation of a dentoalveolar abscess or sinusitis. The abscess may localise and form a fistulous tract (mucosa or skin) or may spread through fascial planes forming abscess collections in potential anatomic spaces (Killey et al., 1975). (Figure 4. A and 4. B) by Chow et al., 1978).

Figure 4. Routes of spread of odontogenic orofacial infections along planes of least resistance.

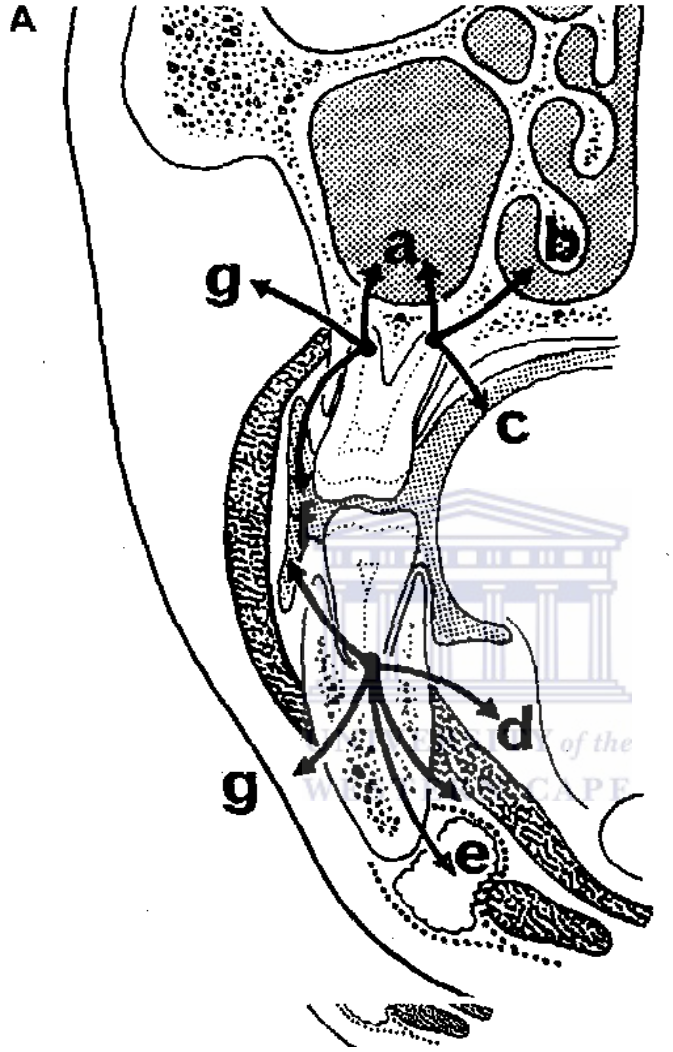

Figure 4. (A) Coronal section in region of first molar teeth.
a = maxillary antrum; **b** = nasal cavity; **c** = palatal plate; d = sublingual space (above mylohyoid muscle); **e** =submandibular space (below mylohyoid muscle);
f = intraoral presentation with infection spreading through the buccal plates inside the attachment of the buccinator muscle: **g** = extraoral presentation to buccal space with infection spreading through the buccal plates outside the attachment of the buccinator muscle (Chow et al., 1978).

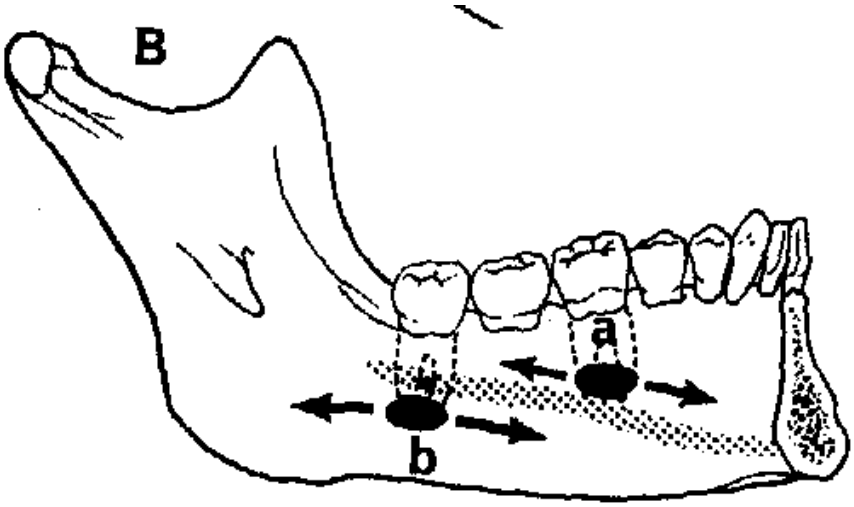

Figure 4. (B).Lingual aspect of the mandible, **a** = apices of involved tooth above the mylohyoid muscle, with spread of infection into sublingual space; **b** = apices of involved tooth below the mylohyoid muscle, with spread of infection into submandibular space (Chow et al., 1978).

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Table 1: Borders of the Deep Spaces of the Head and Neck

	Borders					
Space	**Anterior**	**Posterior**	**Superior**	**Inferior**	**Superficial or Medial***	**Deep or Lateral†**
Buccal	Corner of mouth	Masseter m., pterygomandibular Space	Maxilla, infraorbital space	Mandible tissue and skin	Subcutaneous	Buccinator m.
Infraorbital	Nasal cartilages	Buccal space	Quadratus labii superioris m.	Oral mucosa	Quadratus labii superioris m.	Levator anguli oris m., maxilla
Submandibular	Ant.belly digastric m.	Post. Belly digastric, stylohyoid, stylopharyngeus mm.	Inf.and med. surfaces of Mandible	Digastric tendon	Platysma m., investing fascia	Mylohyoid, hyoglossus sup. constrictor mm
Submental	Inf. border of mandible	Hyoid bone	Mylohyoid m.	Investing fascia	Investing fascia	Ant. bellies digastric m.†
Sublingual	Lingual surface of mandible	Submandibular Space	Oral mucosa	Mylohyoid m.	Muscles of tongue*	Lingual surface of mandible†
Pterygomandibular	Buccal space	Parotid gland	Lateral pterygoid m.	Inf. border of mandible	Med. Pterygoid muscle*	Ascending ramus of mandible†
Submasseteric	Buccal space	Parotid gland	Zygomatic arch	Inf. border of mandible	Ascending ramus of mandible*	Masseter m.†
Lateral pharyngeal	Sup. and mid. pharyngeal constrictor mm.	Carotid sheath and scalene fascia	Skull base	Hyoid bone	Pharyngeal constrictors and Retropharyngeal space*	Medial pterygoid m.†
Retropharyngeal	Sup. and mid. pharyngeal constrictor mm.	Alar fascia	Skull base	Fusion of alar and prevertebral fasciae at C6-T4	—	Carotid sheath and lateral pharyngeal space†
Pretracheal	Sternothyroid-thyrohyoid fascia	Retropharyngeal space	Thyroid cartilage	Superior mediastinum	Sternothyroid-thyrohyoid fascia	Visceral fascia over trachea and thyroid gland

Peterson et al. adapted from Flynn.,2003

ant. = anterior; inf. = inferior; lat. = lateral; m. = muscle; mm. = muscles; med. = medial; mid. = middle; post. = posterior; sup. = superior.
*Medial border; †lateral border.

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Table 2: Relations of Deep Spaces in Infections

Space	Likely Causes	Contents	Neighboring Spaces	Approach for Incision and Drainage
Buccal	Upper bicuspids Upper molars Lower bicuspids	Parotid duct Ant. facial a. and v. Transverse facial a. and v. Buccal fat pad	Infraorbital Pterygomandibular Infratemporal	Intraoral (small) Extraoral (large)
Infraorbital	Upper cuspid	Angular a. and v. Infraorbital n.	Buccal	Intraoral
Submandibular	Lower molars	Submandibular gland Facial a. and v. Lymph nodes	Sublingual Submental Lateral pharyngeal Buccal	Extraoral
Submental	Lower anteriors Fracture of symphysis	Ant. Jugular v. Lymph nodes	Submandibular (on either side)	Extraoral
Sublingual	Lower bicuspids Lower molars Direct trauma	Sublingual glands Wharton's ducts Lingual n. Sublingual a. and v.	Submandibular Lateral pharyngeal Visceral (trachea and esophagus)	Intraoral Intraoral-extraoral
Pterygomandibular	Lower third molars Fracture of angle of Mandible	Mandibular div. of trigeminal n. Inf. Alveolar a. and v.	Buccal Lateral pharyngeal Submasseteric Deep temporal Parotid Peritonsillar	Intraoral Intraoral-extraoral
Submasseteric	Lower third molars Fracture of angle of mandible	Masseteric a. and v.	Buccal Pterygomandibular Superf. temporal Parotid	Intraoral Intraoral-extraoral
Infratemporal and deep temporal	Upper molars	Pterygoid plexus Internal maxillary a. and v. Mandibular div. of trigeminal n. Skull base foramina	Buccal Superf. temporal Inf. petrosal sinus	Intraoral Extraoral Intraoral-extraoral
Superfical temporal	Upper molars Lower molars	Temporal fat pad Temporal branch of facial n.	Buccal Deep temporal	Intraoral Extraoral Intraoral-extraoral
Lateral pharyngeal	Lower third molars Tonsillar infection in neighbouring spaces	Carotid a. Internal jugular v. Vagus n. Cervical sympathetic chain	Pterygomandibular Submandibular Sublingual Peritonsillar Retropharyngeal	Intraoral Intraoral-extraoral

Peterson et al. adapted from Flynn .,2003 a = artery; div. =division; inf. = inferior; n = nerve; superf. = superficial; v = vein.

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The propagation of periapical infection can normally be predicted as it usually follows a typical pattern. In the maxilla, periapical infections associated with the roots of the anterior teeth and the buccal roots of the posterior teeth drain labially and buccally respectively. Infections associated with the palatal roots of the posterior teeth drain palatally. Occasionally, if the root apices are in approximation to the maxillary sinus, sinus spread may occur. In adults, the premolar and molar apices are often inferior to the buccinator muscle attachment, localising the abscess intraorally. In children, the apices are higher in relation (buccinator) and therefore spread superiorly (canine space).

Mandibular incisors or canine infection commonly spread labially (thinner cortex). If apices are superior to the mentalis muscle insertion, it will spread intraorally and if it is below the muscle, extraoral spread is possible. Mandibular premolars and molars often perforate buccally, whereas second and third molars mostly perforate lingually.

Figure 5 illustrates the possible spread of infection to the sublingual and submandibular spaces determined by the relationship of root apices to the mylohyoid muscle.

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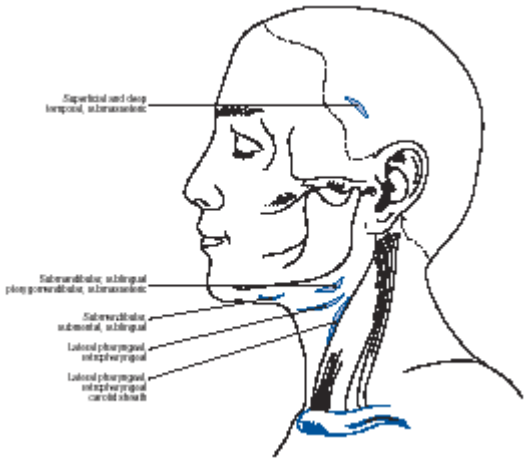

Figure 5. Incision placement for extraoral drainage of head and neck infections. Incisions at the following points may be used to drain infections in the indicated spaces: superficial and deep temporal, submasseteric; submandibular, submental, sublingual; submandibular, sublingual, pterygomandibular, submasseteric; lateral pharyngeal, retropharyngeal; lateral pharyngeal, retropharyngeal, carotid sheath. (Peterson et al. 2003, adapted from Flynn).

2.5 Management

Peterson et al. (2003) describes eight steps in the management of odontogenic infections. This is a thorough, logical and scientifically sound approach and therefore this format will be adopted in the critical analysis of literature in this section.

The eight steps are:

1. Determine the severity of the infection
2. Evaluate host defenses
3. Decide on setting of care
4. Treat surgically
5. Support medically
6. Choose and prescribe antibiotic therapy
7. Administer the antibiotic properly
8. Evaluate the patient frequently

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2.5.1 Determine the Severity of Infection

A history and thorough examination will allow the surgeon to assess the severity of the infection, by assessing the anatomic space/s involved, the possibility of airway compromise, the rate of progression and to what measure systemic reserves and homeostasis is being challenged.

Flynn et al. (1999) created a scoring system to assess the severity of airway compromise. He divided the anatomic locations into low, moderate, severe and extremely severe and scored each from one to four with increasing severity (Table 3).

Table 3: Severity Scores of Fascial Space Infections

Severity Score	Anatomic Space
Severity score = 1 (low risk to airway or vital structures)	Vestibular Subperiosteal Space of the body of the mandible Infraorbital Buccal
Severity score = 2 (moderate risk to airway or vital structures)	Submandibular Submental Sublingual Pterygomandibular Submasseteric Superficial temporal Deep temporal (or infratemporal)
Severity score = 3 (high risk to airway or vital structures)	Lateral pharyngeal Retropharyngeal Pretracheal
Severity score = 4 (extreme risk to airway or vital structures)	Danger space (space 4) Mediastinum Intracranial infection

The severity score for a given patient is the sum of the severity scores for all of the spaces involved by cellulitis or abscess, based on clinical and radiographic examination. Flynn et al.,1999.

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Using this scoring system together with the initial white cell count, Flynn et al. (1999) were able to predict the length of stay in hospital in 66% of cases. The rate of progression would be obtained by history and current examination. The causes that were found to be rapidly progressive would negate a more aggressive management. In an analysis by these authors, they also found that there was a negative correlation between severity and number of days prior to consultation, confirming that rapidly progressing cases presented sooner than slowly progressing cases.

Airway compromise is a major cause of death in severe odontogenic infections. In a study by Williams (1940), a 54% mortality rate was reported, which was later reduced to 10% (Williams and Guralnick, 1943). This was achieved by ensuring airway safety and aggressive surgical drainage of all spaces; this in a pre–antibiotic era.

The mortality rate was reported to be above 4% for Ludwig's angina (Haug et al., 1980). With airway compromise caused by deep fascial space infection, intubating

the patient is extremely difficult despite fibre-optic assisted intubation. Often emergency airway procedures like cricothyroidotomy or tracheostomy are required. This is sometimes done through a septic collection in the neck to secure an airway. Indications for tracheostomy are listed in Figure 6.

Figure 6. Indications for tracheostomy [Pryor et al., 2000]

- To relieve upper airway obstruction
    - Foreign body
    - Trauma
    - Acute infection - acute epiglottitis, diphtheria, Ludwig's Angina
    - Glottic oedema
    - Bilateral abductor paralysis of the vocal cords
    - Tumours of the larynx
    - Congenital web or atresia
- To improve respiratory function
    - Fulminating bronchopneumonia
    - Chronic bronchitis and emphysema
    - Chest injury and flail chest
- Respiratory paralysis
    - Unconscious head injury
    - Bulbar poliomyelitis
    - Tetanus

On clinical examination of a patient with airway compromise, stridor may be heard. The patient may assume an upright or slightly forward posture with chin tilted up, and may gasp or may be unable to breathe when lying flat. It is standard practice in our units to assess all severe cases by sitting the patient upright and slowly lowering the backrest of the chair towards a flat position. By doing this, we can often assess the severity of airway occlusion, where those with impending occlusion will have difficulty breathing in the position slightly tilted back or even in the upright position.

Airway compromise can be assessed via radiographs of the lateral neck and CT scans. A clinical examination may give a good indication of the risk of airway compromise. Severe involvement is seen when the floor of mouth is raised and 'full' (indurated with lifting or without fluctuance, displacing the tongue posteriorly). Other locations of severe airway compromise are found when the tonsillar pillar is edematous and swollen with or without uvula displacement, when the lateral pharyngeal space (lingual surface of mandible posterior to the third molar) is tender or edematous and the patient cannot swallow.

2.5.2 Evaluating Host Defenses

Many diseases and treatments (steroid, radiotherapy, chemotherapy) compromise the immune system. These patients are more prone to infection and often of a more severe nature (Neville et al., 2002). It is therefore recommended that more aggressive management be instituted to facilitate earlier recovery. Figure 7 shows a list of factors associated with immune compromise (Miloro, 2004).

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Figure 7. Factors associated with immune system compromise [Peterson et al. 2003]

- Diabetes
- Steroid therapy
- Organ transplants
- Malignancy
- Chemotherapy
- Chronic renal disease
- Malnutrition
- Alcoholism
- End-stage AIDS

The systemic reserve of the host is determined by the age (limited reserves in young and old), nutritional state, state of health prior to infection, chronosity of infection

and the magnitude of the infection. These are the main factors assisting the host to survive.

2.5.3 Deciding on Setting of Care

Miloro et al. (2004) suggested six factors to warrant hospital admission (Figure 8). The author would also like to suggest the addition of another indication for admission, namely poor systemic reserves. In the developing world, patients with poor systemic reserves often encounted. These patients may develop small infections with no toxic symptoms (not threatening airway), and may not require IV antibiotics. Unfortunately due to poor natural reserves or chronic infections (TB commonly), their recovery will be poor with increased risk of complications. Often, they have to be treated surgically for the complications. With admission on IV antibiotics, their rate of recovery and risk of complications will be reduced. *Comprehensive primary management is better than re-treatment of progressive disease or complications thereof.*

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Figure 8. Indications for Hospital Admission [Miloro et al., 2004]

- Temperature >38.3°C
- Dehydration
- Threat to airway or vital structures
- Infection in moderate or high severity anatomic spaces
- Need for general anaesthesia
- Need for in patient control for systemic disease

Patients with severe illness may be unable to maintain organ or system function. When organ failure occurs, it must be identified (Figure 9) and managed expeditiously. This includes surgical treatment (eliminate cause) and appropriate supportive medical management (high-care or intensive care). Figure 10 provides a brief summary of indications for intensive care admission (McIntosh, 2002).

Figure 9. Indications for ICU Admission

- Mechanical support of organ function
- Respiratory - ventilation / CPAP
- Renal - haemofiltration / haemodialysis
- Cardiac - ECG monitoring and inotropic support
- Hepatic - blood transfusion
- Neurological - intracranial pressure monitoring
- Usually has 1:1 nursing care

Figure 10. Factors Indicating Severe Organ Dysfunction or Pending Organ Failure [McIntosh, 2002]

- Threatened airway
- Respiratory arrest
- Respiratory rate >40 or <8 breaths / min
- Oxygen saturation <90% on >50% oxygen
- Cardiac arrest
- Pulse rate <40 or >140 beats / min
- Systolic blood pressure < 90 mmHg
- Sudden fall in level of consciousness
- Repeated or prolonged seizures
- Rising arterial carbon dioxide tension with respiratory acidosis

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2.5.4 Treat Surgically

Airway security is the primary concern, followed by removal of the cause and definitive drainage. A thorough knowledge of anatomy, appropriately made incisions and blunt dissection will allow complete evacuation of all affected fascial spaces. (Figures 5 and 11).

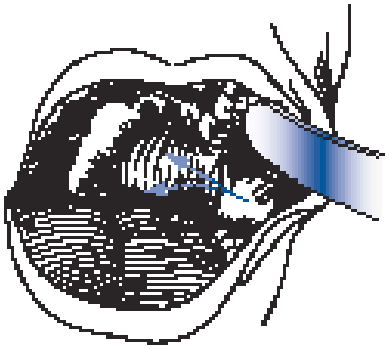

Figure 11. Intraoral incision placement for drainage of the anterior compartment of the lateral pharyngeal space (curved arrow) and the pterygomandibular space (straight arrow). (Peterson et al. 2003, adapted from Flynn).

Incision and drainage should be achieved with the protection of all vital structures and gravity-assisted drainage should be created wherever possible. Incisions should be planned within relaxed tension lines of the neck or face. The use of through-and-through drainage is often used for the provision of two pathways for the evacuation of pus, and efficient, unidirectional flow during irrigation.

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A few decades ago, patients were first placed on antibiotics to 'localise' the abscess prior to extraction and incision and drainage (Piecuch, 1962).

In a landmark study of its time, Krogh (1951), in a sample of 2626, found only a 3% complication rate when the offending tooth was removed at initial presentation. Hall et al. (1968) published a study of 350 patients with odontogenic cellulitis that were divided into two groups. The one group had extractions on the day of presentation, and in the second group, antibiotic treatment was given to 'localise the infection'. The offending tooth was then extracted on the fourth day. The finding was that both groups had no spread of cellulitis, however, the group receiving extractions on the day of presentation recovered more rapidly than the group with delayed treatment. The author also found that the second group (delayed treatment group), was twice as likely to require extra-oral incision and drainage. Therefore, early management not

only gives early relief of symptoms, but a 97% chance of cure as well. The 'watchful waiting' theory telling patients to return when the swelling has gone, is therefore antiquated and without sound basis.

Culture and sensitivity testing, according to Miloro (2004), should not be done for minor oral infections as empiric antibiotic therapy is usually successful. It would be difficult to justify the additional costs of time-consuming microbiological tests when resolution of infection is obtained prior to results (sensitivity). It is generally accepted that microbiological testing is done for infections that are serious enough to warrant admission, or where immune compromise and chronic infections are recalcitrant to therapy.

2.5.5 Support Medically

Medical supportive care is essential with severe odontogenic infections, comprising of hydration, nutrition, and control of fever (Miloro, 2004). This author advocated adding active management for the prevention of deep venous thrombosis (below knee pressure stockings, medical management and early mobilisation), prevention of bedsores (early mobilisation, moisturising skin and good bed hygiene) and prevention of stomach ulceration (early feeding and medical management), all managed according to the risk- benefit (including cost) rationale and assessed individually.

Initial temperature has shown to be a significant predictor of length of hospital stay in severe odontogenic infections (Flynn et al. 1999). It is known that fever below 39.4º C promotes phagocytosis, increases blood flow to affected areas, raises metabolic rate and enhances antibody functioning. Very high fever (> 39.4º C) drives the body to a catabolic state, depleting reserves and making immune and metabolic functions less effective. Therefore, temperature must be managed either medically (paracetamol, aspirin, acetamorphine) or with local measures like cool IV infusions, tepid sponge, fan cooling, chilled drinks and cool baths. Fever also increases metabolic demand by 5% to 8% per degree (Marino, 2007). The replacement of daily fluid needs,

comprising of sensible, insensible and additional losses (vomiting, diarrhoea, fistula), is essential for the maintenance of adequate hydration. The sensible losses, primarily through sweat, are increased by 250ml per degree of fever. Insensible losses, from the lung and skin, increase by 50 to 75ml per degree of fever.

2.5.6 Choose and Prescribe Antibiotic Therapy (Focus on Odontogenic Infections)

Orofacial infections have far-reaching clinical implications due to its nature of rapidly progressive spreading and risk of mortality. Even though we live in an era of antibiotics, we know that this factor alone will not cure the infection (Hall et al., 1968).

The selection of the appropriate antibiotics is determined by pathogen, host factors, and pharmacological factors (reviewed in section 5.7. below).

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2.5.6.1 Pathogenic Factors

This is determined by the type of organisms (refer to causes of infection) responsible for the infection and the organism's resistance to antibiotics. Many authors (Fazakerley et al., 1993; Flynn et al. 1999; Miloro, 2004) have substantiated that mild odontogenic infections (outpatients) can be treated successfully with oral penicillin together with root canal therapy or extraction. However, it was found in studies comparing various antibiotics (clindamycin, amoxyllin, co–amoxiclav), that the recovery at 48 hours was better with the above antibiotic therapy. At seven days, the penicillin faired equally well (Fazakerley et al., 1993; Lewis et al., 1990; Gilmore et al., 1998).

For odontogenic infections requiring hospital admission, more authors advocate the use of antibiotics other than penicillin. In the study by Flynn et al. (1999), he found a 26% failure rate of Penicillin when used empirically in a series of 34 hospitalised

cases. Within this population, 31 patients were given penicillin (three had penicillin allergies). The authors reported that eight of the patients were reported to have 'therapeutic failure'. This was determined by swelling not subsiding, continued fever (> 48 hours) and continued raised white cell counts despite CT verified surgical drainage.

In a second study by Flynn et al. (2006b), they found penicillin resistance in 19% of identified bacterial strains that occured 54% of the population. He found a 'therapeutic failure' (same criteria as 1999 study) in 2%, and re-operation in 8% of cases. In a review article by Robertson and Smith (2009), they found that antibiotic resistance in the acute dental abscess has been increasing with the exception of metronidazole. Their findings showed resistance rates for amoxycillin ranging from 9% to 54%.

The specific bacterial species and their antibiotic resistance profiles will be analysed later in the discussion section. However, a brief description of the mechanisms of antibiotic resistance and the acquisition of these genes are briefly explained.
Four mechanisms of bacterial resistance are known to occur:

1. Inactivation of antimicrobial agent
2. Active elimination of antibiotic
3. Alteration of drugs target size
4. Inability of drug to reach its target size

These mechanisms can only occur if the bacteria have the genes to implement them. Four processes have been identified that enables these mechanisms to occur:

1. Spontantaneous mutation
2. Gene transfer (Bacterial conjugation with transfer of genetic material via plasmids)
3. Bacteriophages (Viruses infecting bacteria can insert genetic material)
4. Mosaic Genes (Bacteria absorb fragments of dead members of related species, gaining genetic material)

2.5.6.2 Host Factors

These factors need consideration in the management of each individual, as it may influence the choice of antimicrobial. These include allergy or intolerance to the antibiotic, immune compromise, previous antibiotic therapy, age and pregnancy.

ii) Allergy Intolerance

Penicillin is the most frequently prescribed antibiotic for odontogenic infection. It is found that between 1 to 10% of patients who initially take penicillin, develop an allergic reaction. Those who do not have an adverse reaction, have a 1% chance of future allergy development upon re-exposure (Craig and Mende, 1999). In this study, it was also noted that 10 to 15% of penicillin–allergic patients exhibited cross–sensitivity to cephalosporins. Severe reactions were reported to be rare (Craig and Mende, 1999).

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ii) Immune System Compromise

Patients with immunosuppression are often unable to mount adequate response to invading pathogens. It is therefore recommended that bactericidal rather than bacteriostatic antibiotics be used. Bactericidal antibiotics arrest vital cell processes such as cell wall synthesis and nucleic acid synthesis (Table 4).

Table 4. Bactericidal and bacteriostatic antibiotics [Flynn and Halpern, 2003]

Bactericidal	Bacteriostatic
β-lactams	Macrolides
penicillins	erythromycin
cephalosporins	clarithromycin
carbapenems	azithromycin
monobactams	Clindamycin
Aminoglycosides	Tetracyclines
Vancomycin	Sulfa antibiotics
Metronidazole	
Fluoroquinolones	

According to Flynn and Halpern (2003), HIV positive subjects responded as well as their HIV negative counterparts in their ability to resist oral bacterial infections until late in the course of the disease. They explained that this was due to the normal B–lymphocyte function, which is mainly responsible for combating bacterial pathogens. These lymphocytes are normal until the end stays of the illness when all white cells are affected. They also stated that fungal and viral infections are prevalent due to the poor cell mediated immunity (T cell). In the study by Flynn et al. (2006a) they had one HIV case and two diabetic cases with sepsis. Although these cases were not severe, they required extensive care.

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iii) Previous Antibiotic Therapy

The use of antibiotics is known to cause selection of resistant organisms. It was also found those individuals who were using antibiotics just prior to or at the time of infection, had a higher percentage of resistance to that antibiotic (Doern et al., 1996).

iv) Age and Pregnancy

Certain antibiotics should not be used in children. Tetracyclines should be avoided in children under the age of 10 due to permanent dental staining. Fluoroquinolones cause chondrotoxity (until puberty) and imipenem poses a seizure risk.

In pregnancy, it is recommended that risk benefit assessments be used if no other alternatives are found (Table 5).

Table 5. **Pregnancy risk categories of selected antibiotics [Flynn and Halpern, 2003]**

Antibiotic	Pregnancy risk category	Pregnancy risk
Penicillins		
penicillin G and V	B	
ampicillin	B	
amoxicillin	B	
amoxicillin/clavulanate	B	
ticarcillin/clavulanate	B	
Cephalosporins		
cephalexin	B	
cefazolin	B	
cefaclor	B	
cefuroxime	B	
cefoxitin	B	
cefotaxime	B	
Carbapenems		
imipenem	C	Spontaneous abortions in monkeys
meropenem	B	
Macrolides		
erythromycin	B	
clarithromycin	C	Fetal defects in mice and monkeys
azithromycin	B	
Antianaerobic		
clindamycin	B	
metronidazole	B	
Fluoroquinolones		
ciprofloxacin	C	Spontaneous abortions in rabbits
moxifloxacin	C	Fetal toxicity in rodents and monkeys
Aminoglycosides		
gentamicin	D	Ototoxicity in human fetuses
tobramycin	D	Ototoxicity in human fetuses
Other		
vancomycin	C	Potential ototoxicity in human fetuses
tetracyclines	D	Intrinsic dental staining
doxycycline	D	Intrinsic dental staining
linezolid	C	Fetal toxicity in rodents
telithromycin	B	

A - No risk (pregnancy studies)
B - Animal studies no risk
C - Animal studies toxicity
D - Evidence of human risk

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2.5.7 Administer the Antibiotic Properly

2.5.7.1 Pharmacological Factors

i) Pharmacodynamics

It is essential that the antibiotic chosen is effective against all the likely pathogens. Included are two tables that reflect the spectrum of the most common antibiotics used in orofacial infections. Refer to Table 6. (Flynn and Halpern, 2003).

The benefit of the correct antibiotics can only be realised if the antibiotic concentration is high enough to be effective in the area of infection (tissue distribution). Clindamycin is the antibiotic that reaches the highest concentration within an abscess, reaching 33% of serum levels (Karsten, 1999), thus rendering it very effective in odontogenic infections. Tetracyclines, clindamycin and fluoroquinolones achieve the best bone penetration. Numerous antibiotics cross the inflamed blood brain barrier (Table 7), but clindamycin, macrolides, aminoglycosides and most cephalosporin are amongst those antibiotics that are not appropriate for managing meningitis associated with orofacial sepsis.

Table 6. Antibiotics of choice for head and neck pathogens [Flynn and Halpern, 2003]

Pathogen	Type	First choice antibiotics	Alternative antibiotics
Actinomyces	+, R, A	Penicillin G or ampicillin	Doxycycline Clindamycin Erythromycin
Bacteroides fragilis	_, R, AN	Metronidazole	Clindamycin Cefoxitin, not cefotetan (DOT) Ampicillin/sulbactam
Clostridium species (except C. difficile)	+, R, AN	Penicillin G F clindamycin	Metronidazole Doxycycline Cephalosporin (1st)a
Clostridium difficile	+, R, AN	Metronidazole p.o.	Vancomycin p.o. Bacitracin p.o.
Eikenella corrodens	_, R, A	Penicillin G or V Amoxicillin Amoxicillin/clavulanate	Fluoroquinolones TMP/SMX (avoid clindamycin) Vancomycin
Enterococcus faecalis (group D streptococcus)	+, C, F	Ampicillin +/- gentamicin (for endocarditis or meningitis) Linezolid	Ampicillin/sulbactam Linezolid
Enterococcus faecium (group D streptococcus: b-lactamase +, aminoglycoside and vancomycin resistant)	+, C, F F	Linezolid + stin/dalfopristin +/- choramphenicol +/- doxycycline	Teicoplanin + aminoglycoside (van B) For some strains: no effective regimen (I.D. consultation)
Escherichia coli	_, R, A	Ticarcillin/clavulanate Cephalosporins Imipenem Fluoroquinolones	Meropenem for central nervous system Aztreonam TMP/SMX Tobramycin
Fusobacterium species	_, R, AN	Penicillin G or V	Metronidazole Clindamycin
Haemophilus influenzae (b-lactamase positive)	_, R, F	Amoxicillin/clavulanate Cefaclor Azithro/clarithromycin	Cefotaxime (if life threatening) Ciprofloxacin TMP/SMX
Klebsiella pneumoniae	_, R, A	Cephalosporin (3rd)* Fluoroquinolones	Tobramycin Ticarcillin/clavulanate Imipenem/cilastatin
Klebsiella pneumoniae (producing extended spectrum b-lactamases: ESBLs)	_, R, A	Imipenem/cilastatin Fluoroquinolones	Meropenem
Pasteurella multocida (eg, dog and cat bites)	_, R, A	Penicillin G Amoxicillin/Clavulanate	Doxycline Cephalosporin (2nd)a TMP/SMX
Peptostreptococcus (and former Peptococcus)	+, C, AN	Penicillin G or V	Clindamycin Doxycline Vancomycin

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Table 6. Antibiotics of choice for head and neck pathogens (continued)

Pathogen	Type	First choice antibiotics	Alternative antibiotics
Black pigmented oral Anaerobes (Prevotella and Porphyromonas)	_, R, AN	Clindamycin	PCN + metronidazole Amoxicillin Cefotetan
Proteus vulgaris (indole +)	_, R, A	Cephalosporin (3rd) Fluoroquinolones	Tobramycin Imipenem Ticarcillin/clavulanate
Pseudomonas aeruginosa	_, R, A	Ciprofloxacin Tobramycin	Aztreonam + ceftazidime Piperacillin + tobramycin Cefepime + tobramycin
Salmonella typhi	_, R, A	Fluoroquinolones Ceftriaxone	Chloramphenicol Amoxicillin TMP/SMX
Serratia marcescens	_, R, A	Cephalosporin (3rd) Imipenem Meropenem Fluoroquinolones	Gentamicin Aztreonam
Shigella	_, R, A	Fluoroquinolones Azithromycin	TMP/SMX + ampicillin
Staphylococcus aureus (methicillin sensitive)	+, C, A	Penicillinase-resistant Penicillin	Cephalosporin (1st)a Vancomycin Clindamycin
Staphylococcus aureus (methicillin resistant)	+, C, A	Vancomycin	Teicoplanin Quinupristin-dalfopristin TMP/SMX (some strains) Linezolid
Staphylococcus aureus (methicillin and vanco mycin resistant)	+, C, A	No effective regimen Try vancomycin F rifampin	Quinupristin/dalfopristin Linezolid
Staphylococcus epidermidis (methicillin resistant)	+, C, A	Vancomycin (+ rifampin + gentamicin for prosthetic valve endocarditis)	Quinupristin/dalfopristin
Staphylococcus epidermidis (methicillin and glycopeptide resistant)	+, C, A	Quinupristin/dalfopristin Linezolid	Vancomycin (high dose) New fluoroquinolones?b (rapid resistance a problem)
Streptococcus pneumoniae (Pneumococcus) (penicillin sensitive)	+, C, A	Penicillin G or V Ceftriaxone Amoxicillin	Cefuroxime, cefipime Imipenem New fluoroquinolonesb
Streptococcus pneumoniae (Pneumococcus) (multiantibiotic resistant, including high-level	+, C, A	Vancomycin + Rifampin	Clindamycin New fluoroquinolones (in vitro)

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Table 6. Antibiotics of choice for head and neck pathogens (continued)

Pathogen	Type	First choice antibiotics	Alternative antibiotics
Penicillin, erythromycin, tetracycline, chloramphenicol, and TMP/SMX)			
Streptococcus pyogenes (b-hemolytic streptococcus)	+, C, A	Penicillin G or V (+ gentamicin if serious group B infection)	Cephalosporin (1st)a Erythromycin
Streptococcus viridans (a-hemolytic streptococcus)	+, C, A	Penicillin G or V	Cephalosporin (1st)a Macrolides
Fungal organisms			
Blastomyces	Fungus	Amphotericin B (for systemic cases)	Itraconazole (if surface) Fluconazole (if surface)
Candida	Fungus	Fluconazole Amphotericin B (for systemic cases)	Nystatin (if surface) Clotrimazole (if surface) Ketoconazole (if surface) Itraconazole (if surface)
Coccidioides immitis	Fungus	Itraconazole	Fluconazole Amphotericin B
Histoplasma	Fungus	Amphotericin B (for systemic or immunocompromised cases)	Itraconazole (immunocompetent) Itraconazole (immunocompromised)
Mucormyces	Fungus	Amphotericin B	Control underlying systemic disease

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Abbreviations: A, aerobe; AN, anaerobe; C, coccus; DOT, distasonis, ovatus, and thetaiotamicron group of B. fragilis

F, facultative; PCN, penicillin; R, rod; TMP-SMX, trimethoprim-sulfamethoxazole.
Data from Gilbert DN, Moellering RC Jr, Sande MA. The Sanford guide to antimicrobial therapy 2002. 32nd edition. New

Park (VT): Antimicrobial Therapy Inc.; 2002.
+ = gram positive.
_ = gram negative.
a Number in parentheses after cephalosporins refers to generations within the cephalosporin family.
b New fluoroquinoles are gati-, gemi-, lero-, moxi-, sparfloxacin.

Table 7. Selected antibiotics and the blood-brain barrier [Flynn and Halpern, 2003]

Cerebrospinal fluid	Antibiotic
Therapeutic levels achieved	**Penicillins**
	ampicillin
	nafcillin
	penicillin G, high dose
	ticarcillin[a]
	piperacillin[a]
	Cephalosporins
	ceftazidime
	cefuroxime
	ceftriaxone
	Carbapenem
	Meropenem[b]
	Fluoroquinolones
	levofloxacin
	ciprofloxacin[c]
	Other antibiotics
	metronidazole
	trimethoprim/ sulfamethoxazole[d]
	vancomycine[e]
	Antifungal drugs
	fluconazole
	flucytosine
	Antiviral drugs
	acyclovir
	foscarnet
	ganciclovir
	zidovudine
Therapeutic levels not achieved	**Cephalosporins**
	cefazolin
	cephalexin
	Aminoglycosides
	Macrolides
	erythromycin
	clarithromycin
	azithromycin
	clindamycin
	Antifungal drugs
	amphotericin
	itraconazole
	Antiviral drugs
	saquinavir
	zidovudine

Data from Gilbert DN, Moellering RC Jr, Sande MA. The Sanford guide to antimicrobial therapy. 32nd edition. Hyde Park (VT): Antimicrobial Therapy, Inc.; 2002.

[a] Levels effective for P. aeruginosa and coliforms may not be reached.

[b] Imipenem is avoided in meningitis because of seizure potential. Meropenem is preferred.

[c] Does not reach adequate cerebrospinal fluid levels for streptococci.

[d] Not adequately effective against Neisseria species and coliforms.

[e] High doses are needed for resistant streptococci.

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ii) Pharmacokinetics

This is the reflection of the potency of an antibiotic and is determined by the minimum inhibitory concentration (MIC). It is usually expressed as MIC_{50} and MIC_{90}. This is the minimum concentration of an antibiotic required to kill 50% (MIC_{50}) or 90% (MIC_{90}) of the pathogens.

In time–dependant antibiotics (β – lactam group and vancomycin), it is essential that the serum concentration is above the MIC for at least 40% of the dosage interval. In concentration–dependant antibiotics (fluoroquinolones and aminoglycosides), the efficacy is dependant on the serum concentration ratio of that antibiotic to kill 50% (MIC_{50}) or 90% (MIC_{90}) of pathogens.

The dosage interval of time–dependant antibiotics is determined by the half-life ($t_{1/2}$) of the drug. For explanatory purposes: the half life of benzyl penicillin (Pen G) is 30 minutes, implying that for every half an hour, 50% of the drug is eliminated from the serum. In 2.5 hours (5 X $t_{1/2}$), only about 3% of the drug remains. Thus, if 2 MU Pen G was administered, the serum concentration after 3 hours (6 X $t_{1/2}$), would be approximately 0.3µg/ml and since the MIC_{90} of S.viridans is 0. 2µg/ml this would mean that 2 MU of Pen G will remain above MIC_{90} for about 75% of the time.

2.5.7.2 Empiric Antibiotics

These are the antibiotics given prior to the availability of culture and sensitivity tests, taking into account the most likely pathogens involved and their antibiotic resistance profiles as well as host factors, pharmacodynamics, pharmacokinetics and cost implications for the patient.

Table 8 by Flynn and Halpern (2003) complies with the analysis of the above literature.

Table 8. Empiric antibiotics of choice for head and neck infections [Flynn and Halpern, 2003]

Type of infection		Empiric antibiotic of choice
Odontogenic infections		
Outpatient		Penicillin Clindamycin Cephalexin (or other first-generation cephalosporin)
	Penicillin allergy	Clindamycin Cephalexin (only if nonanaphylactoid penicillin reaction)
Inpatient		Clindamycin Ampicillin + metronidazole Ampicillin + sulbactam
	Penicillin allergy	Clindamycin Moxifloxacin Cefotaxime (only if nonanaphylactoid penicillin reaction)
Rhinosinusitis		
Acute		Amoxicillin Amoxicillin/clavulanate Cefuroxime Moxifloxacin (over 18 years of age)
	Penicillin allergy	Clarithromycin or azithromycin Telithromycin Moxifloxacin (over 18 years of age)
Chronic		Antibiotics not effective: otolaryngologic consultation
Intubated		Imipenem or meropenem Ticarcillin or piperacillin Ceftazidime + vancomycin Cefepime
Fungal Osteomyelitis of the jaw		Amphotericin B Clindamycin Ampicillin + metronidazole Ampicillin + sulbactam
	Penicillin allergy	Clindamycin Moxifloxacin
Histoplasmosis and blastomycosis		Itraconazole Fluconazole Amphotericin B (systemic or disseminated)
Candidiasis		
Oral, non-AIDS		Fluconazole or itraconazole Nystatin or clotrimazole
Oral, AIDS		Fluconazole or itraconazole Amphotericin B

Data from Gilbert DN, Moellering RC Jr, Sande MA. The Sanford guide to antimicrobial therapy. 32nd edition. Hyde Park (VT):
Antimicrobial Therapy Inc.;2002.

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2.5.8 Evaluate the patient frequently

With empiric antibiotics and adequate surgical treatment, as well as all medical supportive care provided (emotional and psychological, counseling if required), improvement of treatment should be seen in 48 hours (Marino, 2007). The physiological recovery of the patient can be measured by the improvement of symptoms (pain, swelling), inflammatory markers, procalcitonin (CRP has slower response) and an improvement in observations (temperature, heart rate, tachypnoea, urine output) (Marino, 2007).

Failure of improvement in the patient can be attributed to the following:

1. Inadequate surgery
2. Depressed host defenses
3. Presence of foreign body
4. Antibiotic problems
   - incorrect bacterial diagnosis
   - dose of antibiotic too low
   - drug not reaching site
   - antibiotic not administered

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With failure to improve at 48 hours, the patient will require a further complete physical examination. Care should be taken not to overlook other medical conditions like tuberculosis, malaria and bacteremia with early septic shock (established shock SIRS criteria). The Gram stain would have to be reviewed and antibiotic therapy changed accordingly. The patient should also be rescanned for the following: inadequately drained collection, new fascial space collection, distant spread (mediastinitis, cavernous sinus thrombosis and meningitis) and even for foreign bodies. Blood tests should be redone to assess pathophysiology.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

A prospective study was undertaken over a period of four consecutive months in patients with orofacial sepsis who required admission or incision and drainage. Patients were referred to either of the two Maxillo-Facial and Oral Surgery Units in the Western Cape, namely Groote Schuur Hospital or the University of the Western Cape Oral Health Centre (Dental Faculty, part of Tygerberg Academic Hospital complex). The study included written informed consent for treatment and HIV testing from either the patient or his/her legal guardian.

3.2 The Study Sample

The initial study sample consisted of fifty-one patients with orofacial sepsis that required admission or incision and drainage. These patients were referred to the two Maxillo-Facial and Oral Surgery Units in the Western Cape. Six patients were excluded from the sample, as they did not meet the inclusion criteria of the study. Of these, two patients refused to have HIV testing and three were excluded due to the omission of essential laboratory results (MCS or HIV results). The last exclusion was due to an analysis error where the pus swab was incorrectly sent to the cytology laboratory. This left a population **sample size** of **forty-five** cases.

3.3 Inclusion Criteria

1. Any patient with orofacial sepsis requiring admission with incision and drainage was considered.
2. Criteria for the admission to hospital included: any cause of orofacial swelling affecting one or more fascial spaces of the head and neck; impending threat to

vital structures including the airway; fever greater than 38.5°C; patient requiring general anaesthesia; and patient requiring supportive medical therapy.

3. Written, informed consent for treatment and HIV testing was obtained from either the patient or his/her legal guardian (the latter applicable to cases of a minor, patients who were legally unable to give consent due to mental handicap, altered mental state due to illness or substances altering cognitive function). The consent process and procedures were explained to the patient in his/her language of choice.
4. Patients who had microbial analysis of the causative pathogen.

3.4 Exclusion Criteria

1. Patients who refused to participate in the study.
2. Failure to meet any of the inclusion criteria of this study.

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3.5 Treatment Methods

A standardised treatment protocol was used for all patients in this study. A thorough initial consultation was conducted, comprising of history, physical examination, HIV pre-test counseling and blood tests (HIV testing, full blood count, plasma ureum and electrolytes testing). Panoramic dental radiographs were taken, and if not available, left and right oblique views were used. CT scans (with contrast) were considered on admission in the case of airway difficulties or in severe cases where no pus was drained. Patients were treated under local anaesthesia unless there was airway compromise. In the case of children or adults where problems of patient cooperation was anticipated, general anaesthesia was also used.

The patient's skin was prepared with antiseptic swabs prior to any skin incisions. This was followed by incision and drainage of all fascial spaces, via mucosal or 'through-and-through' skin to mucosal incisions in the majority of cases. Pus swabs were

taken from within the opened fascial space, avoiding possible contamination with skin, mucosa or saliva. Radio-opaque corrugated drains were placed in all fascial spaces and secured with sutures to either the mucosa or the skin. These wounds were covered with dry dressings.

Adult patients (no allergy history), received a test dose of 50 000 units, fifteen to twenty minutes prior to the loading dose of 5 million units of intravenous Benzyl penicillin. Thereafter, a six hourly dose of 2 million units of Benzyl Penicillin together with an eight hourly dose of 500mg of intravenous Metronidazole was administered. Patients, who were pregnant and not penicillin-allergic, received the same regimen except that Metronidazole was omitted in the first trimester. Children not allergic to penicillin, received an intravenous test dose of 25 000 units of Benzyl penicillin, fifteen to twenty minutes prior the loading dose of 150 000 units/kg/day in four divided doses, and Metronidazole 7.5mg/kg/8-hourly. Clindamycin was administered in the case of penicillin-allergic patients. In the case of adult patients, 600mg of intravenous Clindamycin was given at six hourly intervals. Children received a dose of 25mg/kg/day in four divided doses.

Blood and pus swabs were sent to the laboratory for appropriate analysis within thirty to forty minutes from being obtained. Tests for full blood count, ureum and electrolytes, HIV and pus swab analysis were sent to the haematology, chemistry, virology and microbiology laboratories respectively.

If, after 48 hours, there was no clinical improvement (temperature, pain, swelling), the gram stains were then critically assessed. The patient would then be thoroughly re-examined and clinically reassessed as to the spread of infection and efficacy of the drainage of previously treated fascial spaces. Other causes would also be considered namely, presence of foreign bodies or other medical illnesses. It was at this point, depending on the severity of the infection or a possible threat to airway, that further blood investigations and/or special investigations e.g. CT scans, would be considered.

Based on these findings, antibiotics could be adjusted, re-drainage of fascial spaces could be considered and interdisciplinary assistance could be sought. A minimum of twice-daily irrigation of drains was mandatory until no pus extrusion from the wound occurred. The drains were serially removed from each of the fascial spaces once pus–free irrigation was achieved. The patient were discharged on oral antibiotics and referred to local day hospitals (closest to the patient's residences) for regular dressings. The patients would return to the unit for follow-up treatment in approximately four to seven days.

3.6 Data Collection

An information capture sheet, collecting all relevant data was formulated (Appendix 1) and recorded in duplicate. The original capture sheet served as the patient's notes and the copy was retained for selective recording on a statistics capture form (Appendix 2). The demographic details of the patient included age, gender, previous medical history (including pre-admission HIV status), medical illness (including diabetes and autoimmune disease), medication, allergies, previous surgical history and social habits (including smoking, alcohol abuse and drug abuse).

The location of the abscess was recorded according to the fascial spaces involved. Any other probable location was also recorded. Possible causes of infection were noted and other forms of infection including necrotising fasciitis and osteomyelitis were recorded. HIV test results and CD4 counts (where known) were recorded. The microbiological test results were entered according to the type of organisms seen with Gram Stain, as well as the type of flora cultured ('normal flora' or 'mixed growth'). If 'no growth' was obtained, it was specified whether antibiotics were administered pre-consultation or not.

Blood test results including full blood counts and urea and electrolytes, were recorded. If the patient exhibited poor clinical improvement, changes in antibiotics

and re-debridement or re-drainage, were considered. In the case of patients who were admitted to hospital, the length of stay was recorded as the number of days admitted.

3.7 Processing of Specimens (Microbiology)

Samples were received at the microbiology laboratory via the hospital's 'tube system'. Upon receipt of these samples, they were processed immediately after logging and labeling thereof. Gram stain, cultivation and identification of microorganisms were conducted using the Department of Health Standard Guidelines of Microbiological Techniques.

The sensitivity of the microorganisms cultured or the selected media (based on the type of infection) were assessed, using the Kirby Baurer Diffusion Method (Appendix 3). The microbiology culture was identified, tabulated and analysed according to the sensitivity tests that were conducted.

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3.7.1 Antibiotics used in Sensitivity Testing

1. Penicillin
2. Clindamycin
3. Cloxacillin
4. Vancomycin
5. Gentamycin
6. Cotrimoxazole
7. Ciprofloxacin
8. Cefuroxime
9. Erythromycin
10. Cefotaxime
11. Coamoxiclav
12. Tetracycline

Sensitivity tests for aerobe and facultative species were carried out according to the type of organism cultured (expected resistance profiles) and standard guidelines of laboratory practice (CSLI Guideline). The above-mentioned antibiotics were tested using either the antibiotic disc method or an automated E-Test Strip Test. With the E-Test strip method, MIC values were generated for each bacterial sample. Intermediate resistance in each of these tests (antibiotic disc method or the E-Test Strip) was specific for each organism and each bacteria and was assessed using the CSLI Guideline. (Antibiotic resistance testing on anaerobic organisms cultures are not done at either Groote Schuur or Tygerberg hospitals).

3.8 HIV Testing

In this study, HIV results were based on two consecutive tests. The first test (Vidas HIV DVO ©) is highly sensitive in order to prevent non-reactive samples testing negative. The second (AxSYM HIV Ag/Ab Combo ©) is more specific in order to prevent false positives. The patients were diagnosed as HIV positive when both of these tests yielded positive results. In the case of the second test being indeterminate, both tests were repeated within two to four weeks from the initial analysis.

The tests used at the laboratories of Groote Schuur and Tygerberg Hospitals were the Vidas HIV DVO © and the AxSYM HIV Ag/Ab Combo © tests. The Vidas HIV DVO © showed a sensitivity of 100% and predicative positive values of 90.4%. (Fierz, Erb, 2003). The AxSYM HIV Ag/Ab Combo © test had a specificity of 99.87% and 100% sensitivity.

CHAPTER 4

RESULTS

Of the fifty-one consecutive patients with orofacial sepsis referred to the Maxillo-Facial and Oral Surgery Units in the Western Cape, six subjects did not fulfill the inclusion criteria (refer to study sample), making this study population a total of forty-five (45) subjects.

Table 9: Gender and HIV Prevalence

Gender	HIV Positive	HIV Negative	Percentage
F	3	16	42.22%
M	8	18	57.78%
Total	11	34	
Percentage	24.44%	75.56%	

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Table 9 is an analysis of the study population according to gender and HIV status (confirmed via blood tests). Analysis of the population showed a fair male predominance (15.56%), with almost a quarter of the population being HIV positive.

Table 10: Age Analysis

Sex	Data	HIV Positive	HIV Negative	Total
F	Count of Study No:	3	16	19
	Count of Age	3	16	19
	Average of Age	27.00	34.25	33.11
	StdDev of Age	5.57	20.31	18.83
	Min of Age	21	2	2
	Max of Age	32	76	76
M	Count of Study No:	8	18	26
	Count of Age	8	18	26
	Average of Age	24.88	31.50	29.46
	StdDev of Age	9.03	9.88	9.94
	Min of Age	11	19	11
	Max of Age	39	52	52
Total Count of Study No:		11	34	45
Total Count of Age		11	34	45
Total Average of Age		25.45	32.79	31.00
Total StdDev of Age		8.02	15.48	14.30
Total Min of Age		11	2	2
Total Max of Age		39	76	76

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Table 10 is an analysis of age, indicating average age with the standard deviation, minimum and maximum ages in relation to gender and HIV status. The average age of the HIV positive group was approximately 7 years younger. The HIV negative group had two subjects with extremes in age variation (youngest subject: 2 years and the oldest being 76 years). Even if these two variables were removed, the average age difference between the two groups remained more than 5 years.

On the first consultation, the patient's history was recorded, each subject was specifically asked about his/her HIV status, autoimmune diseases and allergies. Our findings were that no subjects had any known allergies, two had diabetes and five were aware of their HIV positive status. Of the five HIV positive patients, only one was on anti-retroviral treatment. HIV testing revealed that 55% (six subjects) were unaware of their HIV positive status. Only three patients were aware of their CD4 counts (verified by the study, as the NHLS system is linked to day hospitals). Table 11 reflects the three subjects' CD4 counts according to gender.

Table 11: CD4 T-lymphocyte Counts

Sex	Data	HIV Positive	HIV Negative	Total
F	Count of Study No:	3	16	19
	Count of CD4 count	2	0	2
	Average of CD4 count	308.00		308.00
	StdDev of CD4 count	400.22		400.22
	Min of CD4 count	25		25
	Max of CD4 count	591		591
M	Count of Study No:	8	18	26
	Count of CD4 count	1	0	1
	Average of CD4 count	172.00		172.00
	StdDev of CD4 count			
	Min of CD4 count	172		172
	Max of CD4 count	172		172
Total Count of Study No:		11	34	45
Total Count of CD4 count		3		3
Total Average of CD4 count		262.67		262.67
Total StdDev of CD4 count		293.69		293.69
Total Min of CD4 count		25		25
Total Max of CD4 count		591		591

Table 12: Causes of Sepsis

Causes of Infection	HIV Positive	HIV Negative	Total	Percentage
Teeth	7	25	32	71.11%
Skin	0	1 (Poly trauma)	1	2.22%
Septic fracture	3	4	7	15.56%
Uknown	1	2	3	6.67%
Lose screw post-orif	0	1	1	2.22%
Wire ORIF - became septic	0	1	1	2.22%
Total	11	34	45	100.00%

Table 12 indicates the possible causes of infections diagnosed via clinical history or radiographic examinations. The most common cause was related to teeth with 63.64% and 73.53% in the HIV positive and negative groups respectively. All except two were due to decayed teeth. One was due to pericoronitis and the other was a periodontal abscess. This was followed by septic jaw fractures as the second most common cause with 27.27% and 12% for the HIV positive and negative groups respectively. There were two subjects where the cause could not be identified. One interesting case was an 11-year-old boy who was referred for a facial abscess and only after a thorough history and examination, it was found that he had a septic glans penis following ritual circumcision.

The most common teeth implicated were the mandibular posterior teeth (excluding 3rd molars) with 42.86% and 44.0% in the HIV positive and negative groups respectively. Second most common were the mandibular 3rd molars (Table 13).

Table 13: Most Common Causative Teeth

Teeth (specify)	HIV Positive	HIV negative	Total	Percentage
Maxillary ant teeth	0	2	2	6.25%
Maxillary bicuspids and 1st & 2nd molars	2	2	4	12.50%
Maxillary 3rd molars	0	0	0	0%
Mandibular ant teeth	0	0	0	0%
Mandibular bicuspids and 1st & 2nd molars	3	11	14	43.75%
Mandibular 3rd molars	2	9	11	34.38%
Multiple maxillary primary teeth	0	1	1	3.13%

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Intravenous Penicillin G and metronidazole were administered to all except one subject, as no antibiotic allergies were suspected or reported. This subject was pregnant (first trimester) and had 3 fascial spaces involved (right buccal, right submandibular and right lingual spaces). In this case, metronidazole was omitted. In subjects where infections were caused by unknown etiology (three subjects), where skin organisms (one subject) were involved, or with severe infections like Ludwig's Angina (six subjects), intravenous cloxacillin was added.

Table 14: Fascial Spaces Frequency According to Location

Fascial space	HIV positive	HIV negative	Total	Percentage
Buccal	2 (18%)	14 (41%)	16	**23.88%**
Canine	3 (27%)	6 (18%)	9	**13.43%**
Sublingual	0	6 (18%)	6	**8.96%**
Submandibular	4 (36%)	11 (33%)	15	**22.39%**
Submental	1(9%)	1 (3%)	2	**2.99%**
Superficial Temporal	0	1 (3%)	1	**1.49%**
Submasseteric	3 (27%)	6 (18%)	9	**13.43%**
Retropharyngeal	0	1 (3%)	1	**1.49%**
Periodontal abscess	0	1 (3%)	1	**1.49%**
Post-auricular	0	1 (3%)	1	**1.49%**
Ludwigs angina	1(9%)	5 (15%)	6	**8.96%**

Table 14 indicates the types and number of fascial spaces involved. This table shows the frequencies of the fascial spaces in the HIV positive and negative groups. The percentages indicate the frequency of spaces infected per subject. The percentages in the HIV positive and negative columns added up to greater than 100%, as there was more than one fascial space infected per subject in certain cases (Table 15).

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Table 15: Number of Fascial Spaces per Subject

Number of spaces	HIV positive	HIV negative
1	9 (82%)	22 (65%)
2	1 (9%)	6 (18%)
3	0	1 (3%)
4	0	3 (9%)
5	1(9%)	1(3%)
6	0	1(3%)

Table 15 indicates that only 18% of HIV positive subjects had more than one fascial space involved, and only 9% (one subject) had greater than two spaces infected. This compared well to the HIV negative group where 35% had more than one fascial space involved, and 18% (six subjects) had more than two spaces infected. All the subjects with more than four fascial spaces involved, were diagnosed with Ludwig's Angina (bilateral submandibular, sublingual and submental spaces). Of these five cases (Ludwig's Angina), only one was HIV positive.

In the two cases requiring tracheostomies due to airway compromise, both subjects were HIV negative with no known immune-compromising factors and neither of these subjects had resistant bacteria.

Table 16: Culture Result

Culture	HIV Positive	HIV Negative	Total	Percentage
Mixed growth	1 (9.90%)	3 (8.82%)	4	8.89%
Normal flora	2 (18.18%)	5 (14.71%)	7	15.56%
No growth	0	9 (26.47%)	9	20.00%

Table 16: Mixed growth culture occurs when numerous species overgrow each other on the culture medium, making it difficult to identify the species. Normal flora are commensal bacteria and are considered to be a contaminant on the pus swab. No growth occurred in 26.47% of cases (9 subjects) and presented in the HIV negative group only. A ***CHI-Squared = 3.8814 and with P-Value = 0.0488*** **proving that with a > 95% confidence, 'no growth' is likely to occur in the HIV negative group.**

Table 17: Antibiotics Administered in Cases with No Growth on Culture

Types antibiotics	HIV Positive	HIV Negative
Amoxycillin & Metronidazole		2
Ampicillin & Metronidazole		4
PenG, & Metronidazole		2
Co-amoxiclav & Metronidazole		1

Table 17 is the list of antibiotics administered by the referring clinic or private practitioners prior to the initial consultation at the Maxillo-Facial and Oral Surgery units. From this, it can be deduced that the sterile abscesses drained were penicillin-sensitive in eight out of the possible nine cases. However, due to the extended spectrum of the combination of co-amoxiclav and metronidazole, we could not make any conclusions on the bacteria sensitivity to the first line antibiotic (penicillin).

Table 18: Gram Stain Results

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Gram Stain	HIV Positive	HIV Negative
Gram positive cocci	10 (90.91%)	27 (79.41%)
Gram positive bacilli	6 (54.54%)	22 (64.70%)
Gram negative Cocci	3 (27.27%)	4 (11.76%)
Gram negative bacilli	7(63.64%)	18 (52.94%)

Table 18: Gram positive cocci were the most common bacterial form found in both the HIV positive and negative groups. The second most common group was the Gram negative bacilli for the HIV positive group and the Gram positive bacilli for the HIV negative group with 63.64% and 64.70. These analyses were all reflected in six groups from 0 to 5. With 0 – no bacteria seen; 1 very scanty; 2 - scanty; 3 - moderate; 4 - abundant; 5 – innumerable as reported from laboratory (Table 19).

Table 19: Gram Positive Bacilli

Gram Positive Bacilli	HIV Positive	HIV Negative	Total
1 & 2	5	8	13
3 & 4	1	14	15
Total	6 (54.54%)	22 (64.70%)	28

Table 19 **depicts the Gram Positive Bacilli** analysed according to HIV status, with **a statistically significant larger frequency of Gram Positive Bacilli in the HIV negative group** as proven with *CHI-Squared = 4.1815 and a* ***P-Value = 0.0409.***

Figure 12. Gram Stain for HIV Positive Group

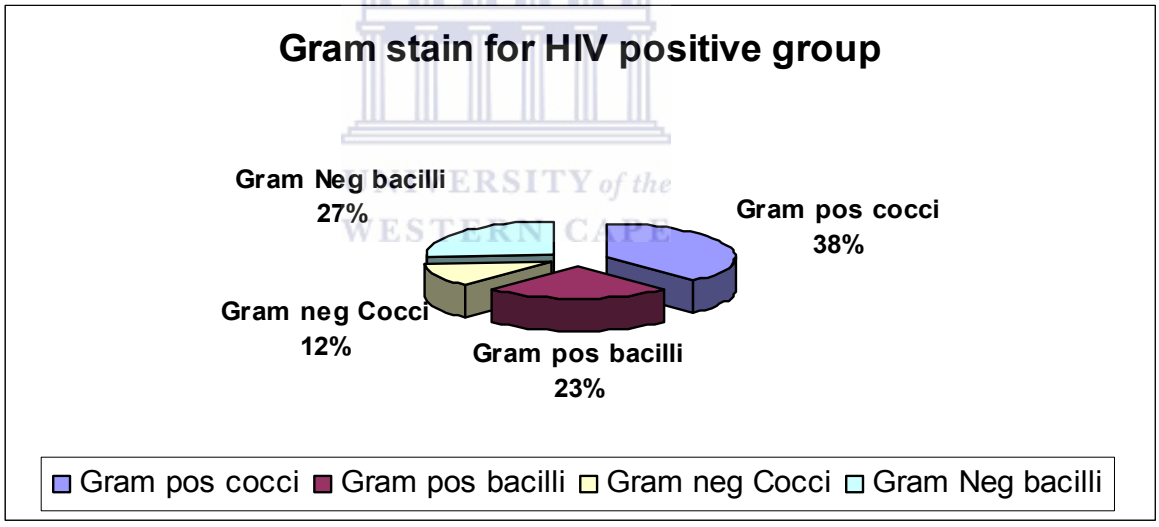

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Figure 13: Gram Stain for HIV Negative Group

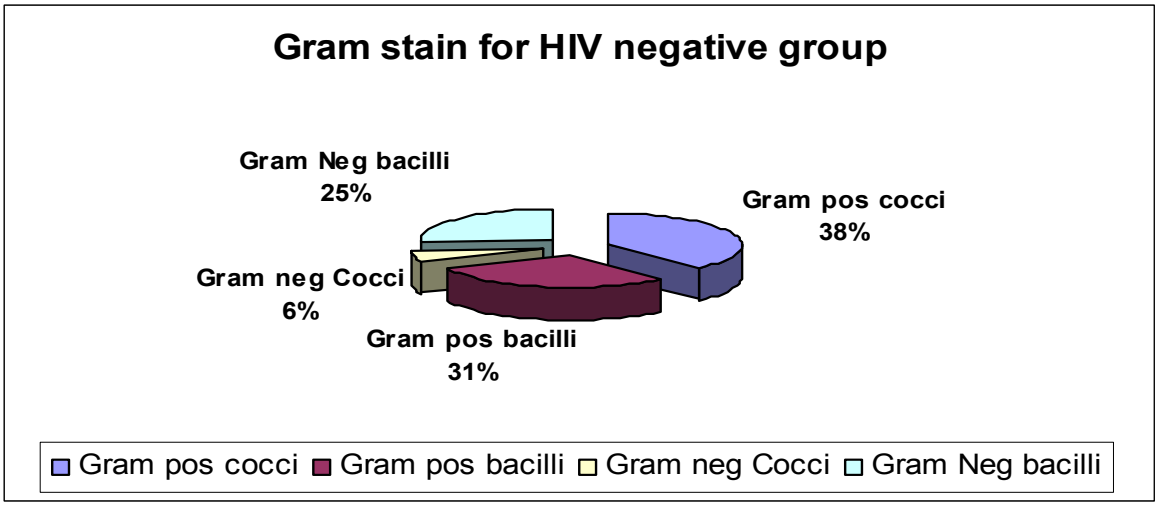

Table 20: HIV Positive Group Depicting Abnormalities in Blood Tests versus the Number of Fascial Spaces Involved

No. of spaces	Haemoglobin	Platelets	Sodium	Potassium	Chloride	Urea	Creatinine
1							
2	11.2	375	135	4.8	95	2.6	65
1							
1			138	4.1	99	2	83
5	15.8	33	140	4		5.2	72
1	15.5	205	128	5	105	3.2	62
1	13.5	329	141	3.2	100	4.2	85
1							
1	9	148	141	4.8	110	3	63
1	12	412	133		104	4.1	
1	14.3	215	137	3.7	101	3	

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Table 20: Abnormalities in blood tests did not seem to correlate with the extent (number of fascial spaces) of the abscesses. However, the HIV positive group (eight subjects with results) showed an alarmingly high number (seven subjects) of abnormalities in blood and electrolyte values.

Analysis of the data comparing electrolyte values in the HIV positive and negative groups, indicated that all the electrolytes (sodium, potassium, chloride, urea and creatinine) did not have any statistical significant differences. However, in the HIV positive group there were many values slightly outside the normal range (according to NHLS laboratory values). Due to the small population size, this was not statistically significant.

The full blood counts showed that the HIV positive females compared to the HIV negative female group had an average white cell count of 7.31 and 13.05 respectively. However, in the male HIV positive versus negative groups, the converse was found, with 21.00 and 14.02 respectively. This paradox could possibly be explained due to the small group of HIV positive females (three subjects) who had advanced stages of HIV. Only one of these subjects was on anti-retroviral medication, despite the low CD4 cell counts of two of the three patients. No CD4 count was available for the last patient. The male HIV positive group consisted of eight subjects; only two subjects were aware of their HIV status and one had a recorded CD4 count of 172. The small size of the female group with more advanced disease, could possibly explain the poor white cell response to the abscesses (Table 21).

Table 21: White Cell Count Comparing HIV Status and Gender

Sex	Data	HIV Positive	HIV Negative	Total
F	Count of Study No:	3	16	19
	Count of White cell count	3	8	11
	Average of White cell count	7.31	13.05	11.49
	StdDev of White cell count	2.87	2.16	3.48
	Min of White cell count	4.17	10.4	4.17
	Max of White cell count	9.8	16.9	16.9
M	Count of Study No:	8	18	26
	Count of White cell count	4	9	13
	Average of White cell count	21.00	14.02	16.17
	StdDev of White cell count	8.52	6.14	7.38
	Min of White cell count	11.2	4.52	4.52
	Max of White cell count	29.4	22.8	29.4
Total Count of Study no:		11	34	45
Total Count of White cell count		7	17	24
Total Average of White cell count		15.13	13.57	14.02
Total StdDev of White cell count		9.62	4.60	6.27
Total Min of White cell count		4.17	4.52	4.17
Total Max of White cell count		29.4	22.8	29.4

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Table 22: Haemoglobin Value in HIV Positive and Negative Groups According to Gender

Sex	Data	HIV Positive	HIV Negative	Total
F	Count of Study No:	3	16	19
	Count of Haemoglobin	3	8	11
	Average of Haemoglobin	12.17	13.03	12.79
	StdDev of Haemoglobin	3.25	2.13	2.34
	Min of Haemoglobin	9	10.8	9
	Max of Haemoglobin	15.5	17.2	17.2
M	Count of Study No:	8	18	26
	Count of Haemoglobin	4	9	13
	Average of Haemoglobin	13.70	14.41	14.19
	StdDev of Haemoglobin	1.92	1.09	1.35
	Min of Haemoglobin	11.2	12.4	11.2
	Max of Haemoglobin	15.8	16	16
Total Count of Study No:		11	34	45
Total Count of Haemoglobin		7	17	24
Total Average of Haemoglobin		13.04	13.76	13.55
Total StdDev of Haemoglobin		2.46	1.76	1.96
Total Min of Haemoglobin		9	10.8	9
Total Max of Haemoglobin		15.8	17.2	17.2

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Table 22 reflects the haemoglobin value in HIV positive and negative groups. There was a lower haemoglobin value in the HIV positive group (both male and female) compared to the HIV negative group. However, it was not statistically significant.

Table 23: Platelet Counts in HIV Positive and Negative Groups According to Gender

Sex	Data	HIV Positive	HIV Negative	Total
F	Count of Study No:	3	16	19
	Count of Platelets	3	8	11
	Average of Platelets	255.00	428.25	381.00
	StdDev of Platelets	138.92	157.19	166.45
	Min of Platelets	148	266	148
	Max of Platelets	412	732	732
M	Count of Study No:	8	18	26
	Count of Platelets	4	8	12
	Average of Platelets	238.00	287.00	270.67
	StdDev of Platelets	152.32	59.02	95.53
	Min of Platelets	33	198	33
	Max of Platelets	375	405	405
Total Count of Study No:		11	34	45
Total Count of Platelets		7	16	23
Total Average of Platelets		245.29	357.63	323.43
Total StdDev of Platelets		134.60	135.93	142.59
Total Min of Platelets		33	198	33
Total Max of Platelets		412	732	732

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Table 23: Platelet counts in the HIV positive groups (both genders) were markedly lower than the negative groups. The average counts for the HIV positive group was 245.29 and for HIV negative group, 357.63, with a difference of 112.34. Population distribution of platelets was illustrated using Side-by-Side Box and Whisker Plot. Statistical analysis, using Chi-Square and Kruskal-Wallis tests both **confirmed a trend**. This result was meaningful, but due to the small population size, was not statistically significant (0.160477).

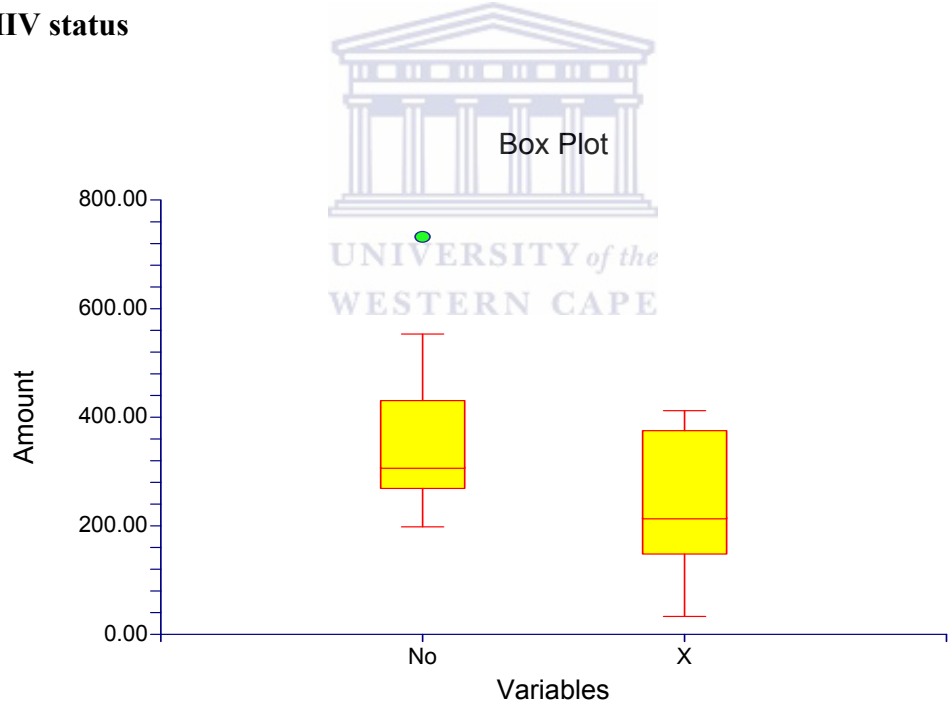
Analysis of Variance Report

Response Platelets

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	2.1621	0.030614	Reject
Kurtosis Normality of Residuals	1.5021	0.133059	Accept
Omnibus Normality of Residuals	6.9309	0.031259	Reject
Modified-Levene Equal-Variance Test	0.0540	0.818522	Accept

Figure 14: Side-by-Side box and Whisker plot illustrating platelet counts according to HIV status



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Side-by-Side box and Whisker plot **X : HIV positive** **No: HIV negative**

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square

A: HIV positive	1	Yes	S(A)	S+sA
S(A)	21	No		S(A)

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: HIV positive	1	61454.47	61454.47	3.34	0.081660	0.415082
S(A)	21	385847.2	18373.68			
Total (Adjusted)	22	447301.7				
Total	23					

* Term significant at alpha = 0.05

Response: Platelets

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Kruskal-Wallis One-Way ANOVA on Ranks

Hypotheses

Ho: All medians are equal.

Ha: At least two medians are different.

Test Results

Method	DF	Chi-Square (H)	Prob Level	Decision(0.05)
Not Corrected for Ties	1	1.96875	0.160581	Accept Ho
Corrected for Ties	1	1.969723	0.160477	Accept Ho
Number Sets of Ties	1			
Multiplicity Factor	6			

Group Detail

	Sum of	Mean

Group	Count	Ranks	Rank	Z-Value	Median
HIV negative	16	213.00	13.31	1.4031	308
X (HIV positive)	7	63.00	9.00	-1.4031	215

Table 24: Length of Stay in Hospital According to HIV Status and Gender

Sex	Data	HIV Positive	HIV Negative	Total
F	Count of Study No:	3	16	19
	Count of Length of stay in hospital	3	16	19
	Average of Length of stay in hospital	3.00	4.38	4.16
	StdDev of Length of stay in hospital	1.00	2.99	2.79
	Min of Length of stay in hospital	2	0	0
	Max of Length of stay in hospital	4	10	10
M	Count of Study No:	8	18	26
	Count of Length of stay in hospital	8	18	26
	Average of Length of stay in hospital	5.00	3.00	3.62
	StdDev of Length of stay in hospital	2.39	1.53	2.02
	Min of Length of stay in hospital	2	0	0
	Max of Length of stay in hospital	8	6	8
Total Count of Study No:		11	34	45
Total Count of Length of stay in hospital		11	34	45
Total Average of Length of stay in hospital		4.45	3.65	3.84
Total StdDev of Length of stay in hospital		2.25	2.40	2.36
Total Min of Length of stay in hospital		2	0	0
Total Max of Length of stay in hospital		8	10	10

Table 24: The average length of admission in hospital was 3.84 days. One was admitted for 10 days and three were not admitted. These three subjects were all healthy individuals between 36 and 39 years old, and did not fulfill the admission

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criteria. Two of these subjects had canine space abscesses and one had a buccal space abscess. Table 24 shows that the HIV positive group's average stay was longer than HIV negative group by almost one day (0.8 of day). Even with a small population, **a trend could be seen**. The length of stay was further analysed, depicting the number of days admitted using a stem and leaves distribution table (Table 25).

Table 25: Length of Stay in Hospital – Stem and Leaves Table

Number of days		Number of days	
HIV positive Stem	**Leaves**	**HIV Negative Stem**	**Leaves**
0		0	000
1		1	
2	22	2	22222 22
3	333	3	33333 33333 33
4	44	4	44
5	5	5	55
6		6	66
7	7	7	77
8	88	8	8
9		9	9
10		10	0
11		11	

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Table 25: A stem and leaves diagram graphically illustrates the length of stay in hospital comparing HIV positive and negative groups. The 'stem' (first) column indicates the number of days admitted in hospital. The 'leaves' (second) column, indicates 1 subject per number on the leaf column according to the number of days admitted (row). This diagram showed that the HIV positive group was predominant in the 2 to 4 day period with a 'tail' in the 7 to 8 day period. Comparing this with the HIV negative group, we found a predominance in the 2 to 3 day period and no

prominent ‘tail’. This population was too small to prove any statistical significance (p <0.005%).

A Box and Whisker plot analysis as well as Kruskal-Wallis test on the length of stay comparing the HIV positive and negative groups, did not prove any statistical significant differences either (refer to the analysis variance report). Analysis of the length of stay for the antibiotic resistant organisms, is dealt with later in this section.

Analysis of Variance Report

Response: Length of stay in hospital

Tests of Assumptions Section

Assumption	**Test Value**	**Prob Level**	**Decision (0.05)**
Skewness Normality of Residuals	2.4359	0.014856	Reject
Kurtosis Normality of Residuals	0.7835	0.433318	Accept
Omnibus Normality of Residuals	6.5473	0.037867	Reject
Modified-Levene Equal-Variance Test	0.0499	0.824275	Accept

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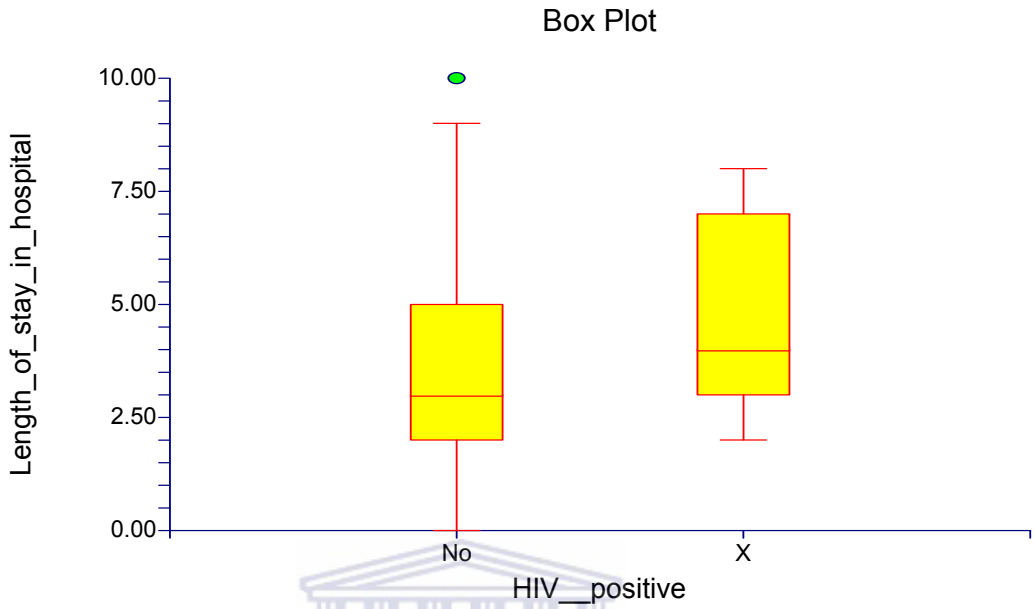
Box Plot Section



Expected Mean Squares Section X : HIV positive No: HIV negative

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: HIV__positive	1	Yes	S(A)	S+sA
S(A)	43	No		S(A)

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: HIV__positive	1	5.419133	5.419133	0.97	0.330451	0.161002
S(A)	43	240.492	5.592837			
Total (Adjusted)	44	245.9111				

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Total	45

* Term significant at alpha = 0.05

Kruskal-Wallis One-Way ANOVA on Ranks

Hypotheses

Ho: All medians are equal.

Ha: At least two medians are different.

Test Results

Method	**DF**	**Chi-Square (H)**	**Prob Level**	**Decision(0.05)**
Not Corrected for Ties	1	1.259881	0.261674	Accept Ho
Corrected for Ties	1	1.332474	0.248366	Accept Ho
Number Sets of Ties	8			
Multiplicity Factor	4962			

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Group Detail

Group	**Count**	**Sum of Ranks**	**Mean Rank**	**Z-Value**	**Median**
HIV negative	34	739.50	21.75	-1.1224	3
HIV positive	11	295.50	26.86	1.1224	4

Table 26: Culture Results Indicating the Frequency and Percentages of Bacterial Types; Percentage and Number of Penicillin and Other Antibiotics that Organisms were Resistant to According to HIV status.

	Number of cases		Percentage of cases		Percentage of pen resistance		No. of other antibiotics bacteria is resistant to							
	HIV postive	HIV negative	HIV postive	HIV negative	HIV postive	HIV negative	0	1	2	3	4	5	6	7
Normal flora	2	5	18	15										
Mixed growth	1	3	9	9										
No growth	0	9		26										
Anaerobes/Facul sp														
Strep viridans group	2	3	25	17.6										
Strep salivarius	0	1		5.9			1							
Strep sanguinis	1	0	12.5		100 Intm			1						
Strep parasanguanis	1	1	12.5	5.9	100 Intm	100 Intm	1	1						
Strep mitis	0	1		5.9										
Strep anginosus	2	6	25	11.8										
Strep constellatus	0	1		5.9			1							
Strep intermedius	1	0	12.5		100 Intm		1							
Strep anginosus	0	4		23.5			4							
Strep melleri	1	1	12.5	5.9	0	0	1, 1							
Other Strepoccoci	4	8	50	47.1										
Strep Thoraltensis	0	1		5.9		100 Intm	1							
Strep gordonii	1	0	12.5				1							
Strep pyogenes	0	1		5.9			1							
Strep cristatus	2	0	25											
Alpha-haemolytic	1	1	12.5	5.9	100	100 Intm	1	1						
β-haemolytic Strep (GF)	0	4		23.5			4							
Strep Pneumoniae	0	1		5.9			1							

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Table 26 (continued)

	Number of cases		Percentage of cases		Percentage of pen resistance		No. of other antibiotics bacteria is resistant to							
	HIV postive	HIV negative	HIV postive	HIV negative	HIV postive	HIV negative	0	1	2	3	4	5	6	7
Other Anaerobes/Facul sp	5	4	62.5	23.5										
Staph aureus	2	1	25	5.9	100	100	2						1	
Klebs pneumonia	2	1	25	5.9	100	100	1, 1	1						
E coli	0	1		5.9		100	1							
Enterobact cloacae	1	1	12.5	5.9	100	100	1, 1							

Number of antibiotics bacteria is resistant to: HIV positive - Pink numbers

HIV negative - Green numbers

Table 26 shows comprehensive information on the causative bacteria, indicating the frequency and percentage according to HIV status. Also shown, is the resistance to penicillin of each bacteria type as well as the number of other antibiotics that these bacteria showed resistance too. In the penicillin resistance column (Percentage of pen resistance), intermediate (Intm) resistance of the bacteria to this antibiotic is also indicated. In this study, only aerobic and facultative anaerobes were cultured (no anaerobes), because according to the Standard Operating Procedure of the NHLS laboratory, pus swabs with specialised carrier mediums are required for anaerobes cultures. This protocol was followed due to the low yields of anaerobes on pus swabs when specialised anaerobic carrier mediums were not used.

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Table 27: Analysis of Penicillin Resistance (Including Intermediate Resistance) Comparing HIV Positive and Negative Groups

Penicillin-resistant cases	HIV Positive	HIV Negative
Resistance	6 bact in 4 cases	4 bact in 4 cases
Intermediate	3 bact in 2 cases	3 bact in 3 cases
Total	9 bact in 5 cases	7 bact in 6 cases

Table 27: In the HIV positive group four cases out of eleven, were resistant to penicillin. An additional two had an intermediate resistance to penicillin. In the HIV negative group, four cases out of thirty-four were resistant to penicillin and only three had intermediate resistance. Due to the small population group, statistical significance ($p<0.005$) could not be proven. However, the trend is quite evident that the **HIV positive group has a greater tendency for resistance to the first line antibiotics.**

Table 28: Resistance to First-line Antibiotics, Indicating Antibiotic Management

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Specify change	HIV Positive	HIV Negative
Clindamycin	1	0
Cloxacillin	1	0
Co-trimoxizole	1	0
Gentamycin	1	0
Vancomycin	0	1

Not changed despite resistant organisms	HIV Positive	HIV Negative
Was on Pen G, cloxacillin & metronidazole	0	2
Pt on Pen G, Metronidazole	0	1

Table 28 indicates the number of subjects resistant to first-line antibiotics (Penicillin G and metronidazole) according to their HIV status. The top section of the table indicates antibiotics changes, and the bottom of the table indicates where antibiotics were not changed. Eight subjects were resistant to the first-line antibiotics. In these subjects (four HIV positive and one HIV negative), antibiotics were changed according to sensitivity results when available. The three subjects who did not have antibiotics changed were HIV negative. Further analysis of this group will be discussed later.

Treatment failures occurred in one case where symptoms and clinical parameters did not improve. A CT scan (with contrast) indicated a retropharyngeal pus collection. The subject was re-operated and new drains were placed. The patient recovered well with no complications.

Table 29 shows that eight subjects out of the total population of 45 subjects (5.63%) were resistant to penicillin. There were ten resistant bacteria (five different types) in eight subjects, averaging 1.25 organisms per subject. Length of admission was not correlated to the number of fascial spaces involved. The average length of admission of the penicillin resistant group (average 5.62 days) was the longest. When subdivided, the HIV positive group with penicillin-resistant organisms had an average length of admission of 5.75 days and the corresponding HIV negative group, 5.50 days.

Three subjects with antibiotic resistance improved despite being on the incorrect antibiotic (all HIV negative). Two subjects' drains were removed, and discharged within 3 days prior to sensitivity results. The last subject was pregnant (first trimester) and had 3 fascial spaces involved (right buccal, right submandibular and right sublingual). Here metronidazole was omitted. She responded well to surgical treatment (temperature returned to normal, symptoms improved, swelling reduced and patient felt well). Her drains were serially removed. However, the submandibular drain continued to produced a little pus for six days, and was only removed on the

seventh day when she was discharged. It was an oversight of the treating surgeons not to have recorded and managed her resistant Enterobacter cloacae (she also had a sensitive β-haemolytic Streptoccocus). She recovered without any complications.

Table 29: Types of Penicillin Resistant Bacteria according to the Number of Subjects, Severity (Number of Fascial Spaces), Antibiotic Change, and Length of Stay also indicating HIV Positive Individuals

Subject number	Bacteria	Changed antibiotic	No. of spaces	Length of stay
1	S aureus	Vancomycin	2	10
11	S aureus	Clindamycin	2	7
14	S aureus	Cloxcacillin	1	5
18	K pneumonia	Gentamycin	5	3
18	Alpha haeml Step	Gentamycin	5	3
40	K pneumonia	Cotrimoxazole	1	8
40	E cloacae	Cotrimoxazole	1	8
			average 2.20	average 6.60

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Subject number	Bacteria	Antibiotic not changed	No. of spaces	Length of stay
7	K. pneumonia	Pen G, Metronidazole	1	3
21	E. coli	Pen G, Metronidazole, Cloxacillin	1	2
30	E. cloacae	Pen G, Metronidazole, Cloxacillin	3	7
			average 1.67	average 4.00

Table totals				
8 subjects	**10 resistant bacteria 5 bacterial types**	**7 antibiotic regimens**	**total 16 average 2.00**	**total 45 average 5.62**

HIV positive cases - Pink numbers

CHAPTER 5

DISCUSSION

5.1 The Study Sample

Within the group of fifty-one subjects with orofacial sepsis who were referred to either of the two Maxillo-Facial and Oral Surgery Units, six subjects did not fulfill the inclusion criteria. Two subjects refused to have HIV testing, three were excluded due to the omission of essential laboratory results (MCS or HIV results) and one subject was excluded due to an analysis error (the pus swab was sent to the cytology laboratory instead of the microbiology laboratory). The methodology used in sample selection was thus free of selection bias. The study sample collected over a 4-month period was representative of the referred population.

The total study population of forty-five subjects comprised of a slightly greater number of males (57.78%) compared to females (42.22%). This finding was verified in other studies (Wood, 1978; Kannangara et al., 1980; Rega et al., 2006; Flynn et al., 2006a).

The age distribution ranged from two to 76 years with an average of 31 years. This wide distribution was in accordance with the findings of other studies (Kannangara et al., 1980; Krishnan et al., 1993; Flynn et al., 2006a). However, in the HIV positive group the average age of 25.45 years was much lower compared to the 32.79 years of the HIV negative group.

In this study, 24.44% of the population was found to be HIV positive. This is significantly higher than the national average of 10.8% as reported by the HSRC Survey 2005 (Key HIV Statistics, 2008). Further analysis of HIV prevalence (according to province), showed that the Western Cape had the lowest rate (15.6%) in reported in a 2006 antenatal study (The South African Department of Health

Antenatal Study, 2006). In this report, it also stated that the antenatal figures were much higher than that of the entire population due to bias of that study sample. This bias was due to the exclusive selection of sexually active female subjects (higher transmission rates of HIV) who had unprotected sex. According to the HSRC Survey in 2005, the total national antenatal HIV prevalence was 29.1%, compared to the average of 13.3% as seen in the entire population of females above two years of age. The average for the entire population of males and females above two years of age was 10.8%, indicating a gross overestimation of antenatal prevalence figures. It is thus evident that the figure of 24.44% of HIV positive subjects in this study is notably higher than the national average of 10.8%.

5.2 Materials and Methods

All subjects were managed according to the same standardized treatment protocol as used at the two Maxillo-Facial and Oral Surgery units. The information was captured on a data capture sheet (Appendix 1) and recorded in duplicate. The original capture sheet served as the patient's notes and a copy was retained for statistical analysis in this study. Variables, including admission core temperature, number of days ill prior to consultation and ethnicity were not recorded. This decision was based on the findings of Flynn et al. (2006a & b) and Amaidas (1990) which showed that the first two variables had no direct correlation to the severity of the infection or length of admission. Ethnicity was not recorded due to the current sensitivity around race categorisation in this country. However, the inclusion of ethnicity as a variable may be of value in further studies, as the burden of numerous diseases which is more prevalent in poorer socio-economic groups (World Health Organisation, 2004), is still largely racially linked in South Africa.

In this study, sterile pus swabs (without a 'carrier medium'), were used for microbiological analysis as per the standard protocol. The sample was obtained from within the wound or from the abscess (in the case of pus evacuation). Within thirty to forty-five minutes after collection, samples were sent (via the hospital's tube system)

to the 'sorting area' and then to the relevant laboratories for processing. Thus, the time from procurement of the sample to laboratory processing, ranged from forty-five minutes to more than sixty minutes. According to the standard protocol of the microbiology laboratories (NHLS), anaerobic cultures were not conducted due the type of pus swab used and the length of time elapsed between sampling and processing (as this methodology produces low yields of anaerobic species). Literature showed that the ideal sampling method would be aspiration of pus via the skin and immediate culture of the sample (Lewis et al., 1990; Brook et al., 1991).

According to Hallander et al. (2005), peri-tonsillar abscesses were sampled by aspiration or via pus swab after incision and drainage. In the study, the authors found that the aspiration method was superior as more anaerobes could be cultured with the least number of contaminants. They also recommended the use of a gassed-out tube as the transport method of choice for septic fluid, especially for anaerobes.

5.3 Causes of Infection

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Odontogenic infections caused 63.64% (seven subjects) and 73.53% (twenty-five subjects) of abscesses in HIV positive and HIV negative individuals respectively. The second most common cause was septic fractures, causing 27.27% and 12% of the infections of HIV positive and HIV negative groups respectively. Most of these septic fractures were possibly caused by odontogenic organisms but due to the overlying soft tissue injuries that sometimes occurred, extra-oral organisms could also have been introduced. Thus, to group septic fractures as odontogenic would not be correct. Amaidas (1990) recorded similar findings, with 56.67% for odontogenic only causes and 21.67% for the tooth in the line of fracture (he grouped these as odontogenic infections with a total of 78.34%). The third most common cause in his study was due to unknown etiology (6.67%). Other studies confirm that odontogenic causes are the most common cause of cervicofacial sepsis (Woods, 1978; Guralnick, 1984; Morey et al., 1984; Boyanova et al., 2006).

The present study showed that the most common teeth causing sepsis were the mandibular bicuspids and the first and second molar teeth with 42.86% (3 subjects) and 44.00% (11 subjects) in the HIV positive and HIV negative groups respectively. The second most common causative teeth were the mandibular third molars with 28.57% and 36.00% in the HIV positive and HIV negative groups respectively. These results compared with the findings of Amaidas (1990) where the third molars accounted for 26.09% and the other posterior teeth accounted for 44.85% of the cases. These findings differed from other studies (Indresano et al., 1992; Storoe et al., 2001; Flynn et al., 2006a) which found the third molars to be the most common causative teeth.

5.4 Spaces Involved

The most common fascial spaces for the HIV positive group were the submandibular spaces (36%), followed by the canine and submasseteric spaces with 27% each (where percentages were indicated as the number of spaces per subject). This differed from the HIV negative group, which had buccal spaces (41%), followed by submandibular (33%) and canine and sublingual spaces with 18% each.

Based on the severity rating by Flynn et al. (2006a), of all the above-mentioned spaces, only the submasseteric space would be rated as a moderate risk (2) to the airway. All the other spaces (submandibular, canine, buccal and sublingual), were classified as low risk (1). Using the severity score of Flynn et al. (2006b), there was almost no difference in the two groups. The author of the present study disagreed with the severity score assigned to the sublingual space due to its common association with Ludwig's Angina. In this study, twelve subjects had sublingual space abscesses, of which six were part of a Ludwig's Angina presentation. According to this study, it may be argued that the submandibular space abscesses (most common in HIV positive group) were more extensive and possibly a more severe form than the more localised buccal space abcesses (most common in HIV negative group).

However, there was no rating system known to this author that could verify this comparison.

The author expected that more spaces would be involved in the medically compromised patients. However, the author was surprised to find that in the HIV positive group only 18% of subjects had more than one fascial space involved compared to 35% in the HIV negative group. Further comparison showed that with the infection of three or more fascial spaces, the HIV positive group had 9% and the HIV negative group had 18%.

In the scientific literature, authors differed about the most common fascial spaces involved. In the study by Amaidas (1990), he listed submandibular (26.51%), body of the mandible (15.53%) and buccal (15.53%) spaces as the most commonly affected spaces. Flynn et al. (2006a), cited the pterygomandibular (60%), submandibular (54%) and lateral pharyngeal (43%) spaces (given as percentage of subjects affected) as the most frequently affected spaces. In the studies by Piecuch (1962) and Levitt (1976), they found that the submandibular and submasseteric spaces were the most common. In some studies, a distinction was made between the most common single space and multiple space infections. In the study by Rega et al. (2006), he found that the most common single space infection was the submandibular space (30%), followed by the buccal space (27.5%); and in the multiple space infections, the submental space was more common than the lateral pharyngeal spaces. According to his article, these findings differed from other literature in that the submandibular space was most frequently seen, followed by the lateral pharyngeal spaces.

In the present study, the average number of spaces of the HIV positive group was 1.45 compared to the HIV negative group with 1.76, perhaps indicating that the spread of infection in the HIV positive group was perhaps not as severe as previously theorised. A comparison of two studies and the number of spaces involved per subject, showed the following: Amaidas (1990): 1.72 spaces and Flynn (2006a): 3.3 spaces (one of the highest reported in literature).

5.5 Blood Analysis

In this study, eight of the eleven HIV positive subjects had blood and electrolyte results that were analysed. Seven of these patients had electrolyte and blood values that deviated from the normal ranges (NHLS values). Analysis of the each electrolyte (sodium, potassium, chloride, urea and creatinine) in the HIV positive and negative groups showed no statistically significant results.

HIV infected subjects develop various cytopaenias that are directly correlated to the degree of immuno-suppression, with thrombocytopaenia possibly being diagnosed most often at the initial presentation (Friel and Scadden, 2009). This may be present at any stage of the HIV infection from asymptomatic infections to end-stage AIDS. In 10% of patients, thrombocytopaenia may be the initial manifestation (Sloand et al., 1992).

In this study, the average values for white cell counts, haemoglobin values and platelet counts were normal in the HIV positive and HIV negative populations. Analysis of the sample according to gender, showed that three female subjects were aware of their HIV status, with two of these subjects in the more advanced stage of the disease (CD4 counts of 25 and 59). In the female HIV positive group, it was found that the white cell response to infection was poor with an average count of 7.31 x 10^9 per litre, compared to the HIV negative group of females with 13.05 x 10^9 per litre. Haemoglobin values of 12.17 and 13.03 were found in the HIV positive and HIV negative groups respectively. The platelet counts of both groups were within normal ranges, with the average platelet count of the HIV positive group at 245.29 compared to the HIV negative group with 357.63. A CHI – Square and Kruskal Wallis tests confirmed a trend ($p = 0.1604$).

5.6 Microbiology

The gram stain showed that gram positive cocci were the predominant microorganisms with a prevalence of 90.91% and 79.4% in the HIV positive and negative groups respectively. The second most common were gram negative bacilli in the HIV positive group and gram positive bacilli in the HIV negative group. A statistically significant larger frequency of gram positive bacilli was seen in the HIV negative group (p-value = 0.0409). According to the literature by Haug (2003) and Robertson and Smith (2009), as well as numerous studies including Amaidas (1990), they found a predominance in gram positive cocci. This finding occurred in studies that did not use the improved methodology of sample collection and laboratory analysis.

The culture results in this study showed the occurrence of mixed growth (15%) and normal flora (56%), which was probably due to contamination during the sampling process (either with cutaneous or oral flora). 'No growth' was found only in the HIV negative group, despite more than 80% of the study population having received antibiotic treatment (from referring practioners) prior to the initial consultation.

A statistically significant result (p = 0.488) showed that (with > 95% confidence) no growth occurred in the HIV negative group only. Eight of the nine subjects with 'no growth' on culture had penicillin-sensitive microorganisms (given penicillin and metronidazole; or ampicillin and metronidazole). One subject was given co-amoxiclav and metronidazole. Due to the extended spectrum of these antibiotics, no conclusion could be drawn about sensitivity of the microflora.

Due to the improvement of sampling, transport media and culturing, studies showed that a mix of anaerobes and facultative anaerobes are predominant in 59 to 75% of cultures (Kuriyama et al., 2000). Only 20% of cultures were strict anaerobes with a small percentage of mixed anaerobic infections (Brook et al., 1991).

Table 30 compares the bacteria found in this study to those by Amaidas (1990), Haug (2003) and Flynnet al. (2006b) as well as the review article by Robertson and Smith (2009). This summary shows that in the present study, there was no culture of anaerobic species. In recent studies conducted in specialist microbiology laboratories, obligate anaerobes outnumbered facultative species by 1.5 - 3:1 (Sakomoto et al., 1998; Khemaleelakul et al., 2002; Baumgartner and Xia, 2003).

S. viridans and anginosus groups were the most common facultative anaerobes. S. aureus was reported as being a frequent coloniser of the oral environment (Smith et al., 2001) with recovery rates of 0.7 to 15 % (Brook et al., 1991; Kuriyama et al., 2002). Higher rates have been reported in children with severe dental abscesses (Brook et al., 1991; Tan et al., 2001). These results compare well with the findings of this study.

According to Brook (2004), Boyanova et al. (2006) and Robertson and Smith (2009), the predominant anaerobic species were Prevotella, Fusobacterium and Porphyromonas. In the study by Boyanova et al. (2006), it was found that the isolation of fusobacterium species was higher ($p < 0.05$) in cases of antibiotic treatment prior to sampling. Further findings in her study showed that 4% of the population cultured Bacteriodes fragilis, which was only reported as an isolated bacterial finding. Clostridia was an infrequently reported pathogen causing infections, either as a sole agent or as part of mixed flora (Robertson and Smith, 2009). In the review by Robertson and Smith, they found that Clostridia species have been identified in a few studies ranging from two to 20% of isolates.

Table 30: A Literature Comparison of Bacteria Causing Cervicofacial Sepsis

Bacteria	3-year Retrospective Study (n=60) Amaidas, 1990	Review Haug, 2003		3-year Prospective Study (n=37) Flynn et al. 2006	Review Robertson and Smith 2009	This Author's Study 2009	
		1980	1990			HIV +ve	HIV -ve
Streptococcus viridans	Total Strep: 39%	26%	21%	milleri - 50% & viridans 8%	MF Facultative Anaerobes	25%	17.6%
Streptococcus anginosus						25%	11.8%
Beta-haemolytic Strep		12%	18%			0%	23.5%
Peptostrepcoccus				50%			
Other Streptococci				13%		50%	23.5%
Staphylococcus Aureus	14%	6%	7%		0.7 - 15% (> in children)	25%	5.9%
Staphylococcus Epidermis	7.6%	8.3%	16%		4% - 65%		
Other Aerobes/ Facultative Species				50%			
Escherichia coli	Gram -ve bacilli: 25%	1.6%				0%	5.9%
Klebsiella		2.2%	2.6%			25%	5.9%
Enterobacter		1.6%	0.8%			12.5%	5.9%
Bacteriodes (β-lactamase+ve)	Bacteriodes: 16%		5.2%			No Anaerobic Testing Performed	
Bacteriodes (β-lactamase–ve)			3.4%				
Bacteriodes fragilis		0.5%	0.8%				
Porphyromonas species	Gram -ve Species: 10%	*	Not reported	63%	MF Facultative Anaerobes Most sudies: Prevotella > Porphyromonas by 10 - 87%		
Prevotella species		**	**				
Clostridium					Not common 2 - 20 % pure/mixed culture		
Eikenella corrodens		7.2%	2.6%				
Haemophilis influenza		4.4%	1.7%				
Fusobacterium nucleatum				21%			
Other Anaerobic species	1.67% Exclusive Anaerobes not classified			88%			
Other Gram –ve species			5.2%		1%		

Key: MF = Most Frequent; * = 15 % Bacteriodes melaninogenicus (nomenclature change to Porphyromonas & Prevotella - Haug, 1980); ** = β lactamase proucers in new nomenclature forms about 50% of Prevotella species.

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5.7 Newer Techniques: Molecular Techniques to Identify Flora

The identification of flora was carried out using two molecular techniques. The one technique used molecular cloning and sequencing to identify organisms using 16srRNA or rDNA on uncultivatable organisms. The second method utilised PCR or DNA-DNA hybridisation checkerboard techniques with species-specific primers of the specific microbes. This has led to the identification of organisms not usually identified such as Treponema species. In a study by Siqueira and Roccus (2004), T. denticola species were found in 79% of dental abscesses. Other organisms also identified were, a Gram positive rod, Bulleidia extructa, Cryptobacterium curtum and Mogibacterium timidum using these non culture techniques (Robertson and Smith, 2009).

5.8 Resistance to Antibiotics

In this study, there were eight subjects with 10 strains of bacteria (5 different types) resistant to penicilin. Within the HIV positive group, four of the eleven subjects (36.36%) were penicillin-resistant and two had differing species of resistant organisms in the same individual. Of the thirty-four HIV negative subjects, there were four individuals (11.76%) who had 4 bacterial strains resistant to penicillin.

This study also found bacterial strains with intermediate resistance to penicillin. The HIV positive group had three bacterial types in two subjects compared to the HIV negative where three bacterial types were found in three subjects. In summary, there were nine bacterial strains that showed resistance and intermediate susceptibility to penicillin as seen in five of the eleven HIV positive subjects (45.45%). In the HIV negative group, there were seven bacterial strains found in seven of the thirty-four subjects (20.59%). Due to the small population size of this study, the correlation between the HIV positive population and its higher penicillin-resistant profile can only be observed as a trend and not a significant finding.

Table 31 shows the percentage of bacterial resistance according to HIV status for penicillin and the number of other antibiotics that these bacteria are resistant to.

Table 31: **Resistance Profile of Bacteria to Penicillin and Other Antibiotics**

	Percentage of penicillin resistance		No. of other antibiotics that bacteria are resistant to						
	HIV postive	HIV negative	0	1	2	3	4	5	6
Alpha haemolytic	100	100 Intm	1	1					
Staph aureus	100	100	2						1
Klebs pneumonia	100	100	1,1	1					
E coli		100	1						
Enterobact cloacae	100	100	1,1						

HIV positive - Pink
HIV negative - Green
Intm = Intermediate Resistance

The most common resistant bacteria identified were Klebsiella pneumonia and S. aureus as found in three subjects each. This was followed by β-haemolytic Streptococcus and E. cloacae, as identified in two subjects each, and E. coli was found in one subject. Of the three subjects with K. pneumonia, two were HIV positive and penicillin-resistant. The other HIV positive subject showed resistance to co-amoxiclav as well. Statistical analysis in this study proved that K. pneumonia was more likely to occur in the HIV positive population (CHI Squared = 1.1675 and p-value = 0.2799). Of the three subjects with S. aureus, two (HIV positive group) were resistant to penicillin, and one (HIV negative group) was resistant to cloxacillin, gentamycin, ciprofloxacin, erythromycin, clindamycin and co-trimoxazole with only sensitivity to vancomycin. Two alpha-haemolytic Streptococcus cases were identified, with one penicillin-resistant subject (HIV positive group) and one with intermediate resistance to penicillin (HIV negative group). Two cases (one HIV positive and one HIV negative), showed penicillin-resistance to E. cloacae, with both cases also showing resistance to co-amoxiclav, and one case (HIV negative group) demonstrated an intermediate resistance to cefuroxime.

E. coli was found in one subject (HIV negative group) and was only resistant to penicillin.

According to the review article by Robertson and Smith (2009), resistance for S. anginosus is rare with 2.3% having a penicillin MIC greater than $1mgl^{-1}$ and for the S. viridans group, this is about 15.5%. In the studies by Kuriyama et al. (2002) and Rega et al. (2006), it was found that the viridans group had a susceptibility to penicillin of 77% and 87.1% respectively. When this organism was tested against other antibiotics for susceptibility, the following was reported: ampicillin (85% and 98.6%), cefazolin (96% and 100%), clindamycin (77% and 98.6%), erythromycin (77% and 83.4%), levofloxacin (92% and 98.6%) for these authors respectively.

According to Haug (2003), S. aureus was the most frequently identified resistant pathogen. In the study by Rega et al. (2006), Staphylococci susceptibility to penicillin was 27.3%, ampicillin 41.2%, cefazolin 70%, ciprofloxacin 95%, clindamycin 89.5%, erythromycin 75%, levofloxacin 84.2% and vancomycin 100%. In the study by Kruke et al. (2008), assessing the antibiotic resistance of S. aureus in cutaneous abscesses of HIV positive patients, he found that 93.5% were penicillin-resistant, 87% oxacillin-resistant (by definition methicillin-resistant S. aureus), cefazolin 84.4%, erythromycin 84.4%, and ciprofloxacin 52.2%. All were susceptible to co-trimoxazole, rifampicin and vancomycin.

In the study by Boyanova et al. (2006), the resistance rates of gram negative anaerobes to amoxicillin was 32% and 13.5% for clindamycin as compared to the study by Paparaskevas et al. (2005), with a resistance rate of 81.8% for penicillin and 31.1% for clindamycin. Other studies showed values intermediate to these (Kuriyama et al., 2000; Aldrige et al., 2001; Kuriyama et al., 2001). Boyanova et al. (2006) also found that the resistance rates of gram negative anaerobes to metronidazole was 2.5%. The most common of these gram negative anaerobes was Prevotella (Robertson and Smith, 2009; Boyanova et al., 2006, Kuriyama et al., 2001). Penicillin resistance for Prevotella

species ranged from 21.7% (Boyanova et al., 2006) to more than 50% (Jousimies-Somer et al., 1999).

The study by Boyanova et al. (2006) found that in patients treated with β-lactam resistant antibiotics, there was a resistance rate of 34% as compared to the rate of 14.3% in the case of patients who did not receive the antibiotics. In this study, the combination of co-amoxiclav and metronidazole showed a resistance rate of 1.3% to gram negative anaerobes. Ampicillin with sulbactam and amoxicillin with clavulanate were the most active antibiotics against anaerobes (Kuriyama et al., 2000). There were some strains of Peptostreptococcus and bacteriodes fragilis that showed resistance rates of 5 to 8% for amoxicillin and clavulanate (Aldrige et al, 2001).

5.9 Length of Stay

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The average length of admission for the population in this study was 3.84 days. In the HIV positive group, the average length of stay was 4.45 days and in the HIV negative group, it was 3.65 days.

Analysis of the penicillin-resistant group (eight subjects), showed an average length of admission of 5.62 days with 5.75 days for the HIV positive subjects and 5.50 days for the HIV negative group. In the study by Flynn et al. (2006 b), he found that subjects with penicillin treatment failure had an increased length of admission ($p = 0.01$). (Penicillin treatment failure was due to toxic or allergic reactions, or the development of necrotising fasciitis, or the failure of clinical improvement 48 hours after incision and drainage.

In the present study, as well as the study by Flynn et al. (2006 b), no correlation was found between deep fascial spaces and the length of admission. Flynn also found using linear regression statistical testing, that a longer length of admission was associated with the following predictor variables; patients who required re-operation had ($p = 0.01$), Severity score ($p = 0.001$), number of infected spaces ($p = 0.001$) and time spent in

operating room (p = 0.007). Other studies by Dodson et al. (2001), Peters et al. (1996), Haug (2003) as well as Flynn et al. (2006 b), found that white cell count, temperature and immune compromise correlated with increased length of stay. The immune compromise described in the above studies mainly consisted of medical illnesses with only isolated HIV cases reported.

In this author's study, using Speerman Rank Correlation, it was found that resistant S. aureus and sensitive or resistant E. cloacae, were positively related to increased length of admission (0.3444). Using the same correlation for S. parasangaunis, a negative relation to length of admission was found for sensitive and intermediate resistance (0.3037). This finding suggests that with non-resistant S. parasangaunis infections, the admission time would be shorter.

CHAPTER 6

CONCLUSIONS

Based on the findings in this study at the two Maxillo-Facial and Oral Surgery units in the Western Cape, the following conclusions can be drawn:

1. Orofacial sepsis has a fair male predominance of 15.56%.
2. The average age (31 years) of the population (young adults) is in accordance with other literature. However, the HIV positive group has a much lower average (25. 5 years).
3. The percentage (24.44%) of HIV positive subjects with orofacial sepsis was higher than the national average (10.8%). (The Western Cape is the province with the lowest prevalence rate).
4. The most common cause of infections was from odontogenic (71.11%). Septic jaw fractures were the second most common cause (15.56%).
5. Mandibular posterior teeth (excluding 3rd molars) were the most common cause of odontogenic infections (43.75%) and mandibular 3rd molars were the second most common cause (44.38%).
6. The most common fascial space affected in the HIV positive group was the submandibular space (36%), followed by the submasseteric and canine spaces (27% each). This compared to the HIV negative group with the buccal space (41%), followed by the submandibular space (33%). Thus, it appears that the HIV negative group may have more localised infections.
7. The HIV positive group who had more than one fascial space involved comprised of 18% of the subjects; one patient was diagnosed as Ludwig's Angina. The HIV negative group had 35%, of which five subjects developed Ludwig's Angina (two required emergency tracheotomies).
8. The average number of spaces involved per subject showed that the HIV positive group had 1.45 compared to the HIV negative group with 1.76

spaces infected. This result showed that contiguous spread of infection (number of spaces) in HIV positive subjects was not more severe.

9. Gram stain results showed gram-positive cocci as the most dominant flora in both HIV positive and negative groups. Gram positive bacilli were statistically significantly more numerous in the HIV negative group (CHI Squares Test: $p = 0.0409$).
10. Pre-treatment with antibiotics (by referring practioners) was associated with sterile infections (abscesses) in 20% of the population. No growth occurred in the HIV negative population with > 95% confidence interval (CHI Squares Test: $p = 0.0488$).
11. In the HIV positive group, there was no correlation between number of spaces infected and electrolyte abnormalities.
12. The average platelet count of the HIV positive group was 245.29 and the HIV negative group was 357.63. A trend was noted with HIV positive subjects having a lower platelet count.
13. The average length of admission in hospital for the population was 3.84 days. The average length of admission for the HIV positive group was 4.45 days and for the HIV negative group, it was 3.65 days. In this study, the average number of spaces affected in the HIV negative group was greater but the average length of stay was shorter.
14. The length of admission significantly correlated with penicillin-resistance (Wilcoxin Rank Sum Test: $p = 0.0072$).
15. Resistant S. aureus and sensitive or resistant E. cloacae were positively related (using Speerman Rank Correlations) to an increased length of admission. A decrease in length of admission was found for sensitive and intermediate resistant S. parasangaunis (0.3037) infections.
16. Penicillin resistance occurred in 17.78% (eight subjects) of the population and with five species of bacteria (10 strains).
17. Of the eight subjects with penicillin-resistance, five were also resistant to co-amoxiclav.

18. It was interesting to note that no subjects were found to be allergic to penicillin.
19. The most common resistant bacteria was S. aureus and K. pneumonia. One MRSA was found and was susceptible to vancomycin.
20. The use of culture and sensitivity tests was essential for the selection of appropriate antibiotics especially when faced with an increasing resistance profile and more severe infections.
21. Cervicofacial infections are polymicrobial in nature and according to the literature, anaerobic organisms dominate with their own resistance patterns (not tested in this study).
22. Due to the high percentage of bacterial resistance to penicillin and co-amoxiclav, it may be necessary to reconsider our current antibiotic regime.
23. A greater number of cervicofacial infections were seen in HIV positive subjects relative to their population prevalence rates. Added to this, was the higher rates of antibiotic resistance and longer hospital admissions found in these subjects. These findings may warrant further investigation of the relationship between HIV positive and negative groups with regard to cervicofacial sepsis.
24. It is the opinion of the author that orofacial sepsis is seen more commonly in HIV infected subjects in the Western Cape. This population has greater numbers of resistant organisms and is more prone to complications.

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REFERENCES

Adekeye, E.O., Cornah, J. 1985. Osteomyelitis of the jaws. A review of 141 cases. Br J Oral Maxillofac Surg. 23: 24-35.

Aldrige, K.E., Ashcraft, D., Cambre, K., Pierson, C.L., Jenkins, S.G. and Rosenblatt, J.E., 2001. Multicentre survey of changing in-vitro antimicrobial susceptibilities of clinical isolates of Bacteroides fragilis groups, Prevotella, Fusobacterium, Porphyromonas and Peptostreptococci species. *Antimicrob Agents Chemother*, 45: 1238-1243.

Amaidas, V.D., 1990. *Cervicofacial infections. A three year retrospective study*. Mmed MFOS thesis. Department of Library and Information Science, University of the Western Cape.

Balcerak, R.J., Sisto, J.M. and Bosack, R.C., 1998. Cervicofacial necrotising fascitis: report of 3 cases and literature review. *J Oral Maxillofac Surg*, 46: 45–9.

Bamberger, D.M., 1991. Antimicrobial treatment of sinusitis. *Semin Respir Infect,* 6: 77– 84.

Bartlett, J.G. and Gorbach, S.L., 1976. Anaerobic infections of the head and neck. *Otoloryngic Clinics of North America*, 9 (3): 655–678.

Baumgartner, J.C. and Xia, T., 2003. Antibiotic susceptibility of bacteria associated with endodontic abscesses. *J Endod*, 29: 44–47.

Boyanova, L., Kolarov, R., Gergova, G., Deliverska, E., Madjarov, J., Marinov, M. and Mitov, I., 2006. Anaerobic bacteria in 118 patients with deep-space head and neck infections from the university hospital of maxillofacial surgery, Sofia, Bulgaria. *J Med Microbiol*, 55: 1285-1289.

Brook, I., 1987. Microbiology of abscesses of the head and neck in children. *Ann Otolrhinolaryngol*, 96: 429–32.

Brook, I., 2004. Microbiology and management of peritonsillar, retropharyngeal, and paraphyryngeal abscesses. *J Oral Maxillofac Surg*, 62: 1545-1550.

Brook, I., Frazier, E.H. and Gher, M.E., 1991. Aerobic and Anaerobic Microbiology of Periapical Abscesses. *Oral Microbiology Immunology*, 6: 123–125.

Butler, K.M. and Baker, C.J., 1992. *Cervical lymphadenitis in paediatric infectious diseases*, 220–230.

Chavez de Paz, L.E., 2007. Redefining the persistent infection of root canals: possible role of biofilm communities. *J Endod*, 33: 652-662.

UNIVERSITY of the WESTERN CAPE

Chow, A.W., Roser, S.M., Brady, F.A. 1978. Orofacial odontogenic infections. Ann Intern Med, 88: 392-402.

Clarke, J.H., 1999. Toothaches and death. *J Hist Dent*, 47: 11–13.

Cleary, K.R. and Batsakis, J.G., 1995. Mycobacterial disease of the head and neck: current perspectives. *Ann Otolrhinolaryngol*, 104: 830–33.

Cotton, M.F., Wasserman, E., Smith, J., Whitelaw, A. and Zarh, H., 2008. High incidence of antimicrobial resistant organisms including extended spectrum beta-lactamase producing Enterobacteriaceae and Methicillin-resistant Staphalococcus aureus in nasopharyngeal and blood isolated of HIV-infected children from Cape Town, South Africa. *BMC Infectious Diseases*, 8 (40): 2334-2338.

Craig, T.J. and Mende, C., 1999. Common allergic and allergic-like reactions to medications: when the cure becomes the curse. *Postgrad Med*, 105: 173–181.

Crum-Cianflone, N., Hale, B., Burgi, A., 2006. Increasing rates of community-acquired MRSA infections among HIV-infected persons. Abstract MOAB0304. Presented at: *The XVI International AIDS Conference, August 13-18 2006 Toronto.*

Dodson, T.B., Barton, J.A. and Kaban, L.B., 2001. Predictors of outcomes in children hospitalised with Maxillo-Facial infections: A linear logistic model. *J Oral Maxillofac Surg*, 49: 838.

UNIVERSITY of the
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Facklam, R., 2002. What happened to the streptoccoci? Overview of taxonomic and nomenclature changes. *Clin Microbiol Rev*, 15: 613–630.

Falkler, W.A.Jr, Enwonu, C.O. and Idigbe, C.O., 1999. Microbiological understandings and mysteries of noma (concrum oris). *Oral Dis*, 5: 150–155.

Fazalerley, M.W., McGowan, P., Hardy, P., Martin, M.v. 1993. A comparative study of cephradine, amoxycillin and phenoxymethylpenicillin in the treatment of acute dentoalveolar infection. Br Dent J, 174: 359-63.

Fierz, W. and Erb, P., 2003. Conference on Retroviruses and Opportunistic infections. *Abstr 10th Conf Retrovir Oppor Infect Feb 10-14 2003 Hynes Conv Cent Boston Mass USA*: Abstract no. 663.

Flynn, T.R. and Halpern, L.R. 2003. Antibiotic selection in head and neck infections. Oral Maxillofacial Surg Clin Am, 15: 17-38.

Flynn, T.R., Shant, R.M., Levi, M.H., Adamo, A.K. and Kraut, R.A., 2006 (a). Severe odontgenic infections, Part I: Prospective report. *J Oral Maxillofac Surg*, 64 (7): 1093 – 1103.

Flynn, T.R., Rabie, M.S. and Hayes, C., 2006 (b). Severe odontogenic infections, Part 2: Prospective outcomes study. *J Oral Maxillofac Surg*, 64 (7): 1104-1113.

Flynn, T.R., Wiltz, M., Adamo, A.K., 1999. Predicting length of hospital stay and penicillin failure in severe odontogenic infections. *Int J Oral Maxillofac Surg*, 28 (l): 48.

Friel, T.J. and Scadden, D.T., 2009. *Hematological manifestations of HIV infections: Thrombocytopenia and other coagulation abnormalities* [online]. Available from: http://www.uptodate.com/patients/content/topic.do. [Accessed 9 April 2009].

Gilmore, W.C., Jacobus, N.V., Gorbach, S.L. 1988. A prospective double-blind evaluation of penicillin versus clindamycin in the treatment of odontogenic infections. J Oral Maxillofac Surg, 46: 1065-70.

Goumas, P.D., Naxalis, S.S., Papavasiliou, D.A., Moschovalis, E.D., Tsintsos, S.J. and Skoutelis, A., 1997. Periapical abscess: causal bacteria and antibiotic sensitivity. *J chemother*, 9: 415–419.

Guralnick, W., 1984. Odontogenic Infections. *British Dental Journal*, 156: 440–447.

Gwaltney, J.M., 2000. Sinusitis. *In*: G.L. Mandell, J.E. Bennett and R. Dolin, eds. *Principles and practice of infectious disease*. 5th edition. Philadelphia: Churchill-Livingstone, 676–686.

Hall, H.D., Gunter, J.W., Jamison, H.C. and McCullum, C.A., 1968. Effect of time of extraction on the resolution of odontogenic cellulitis. *Journal of the American Dental Association*, 7: 626–631.

Haug, R.H., 2003. The Changing Microbiology of Maxillo-Facial Infections. *Oral and Maxillofac Surg Clin N Am*, 15: 1-15.

Haug, R.H., Liu, T.C. and Chen, P.R., 1986. Deep neck infections: Analysis of 185 cases. *Head Neck*, 26: 854.

Haug, R.T., Fitzgerald, B.E., Latta, J.E. and Zallen, R.D., 1980. Ludwig's angina: report of two cases and review of the literature from 1945 to January 1979. *J Oral Surg*, 38: 849.

Hallander, H.O.,Flodstrom, A., Holmberg, K., 1975. Influence of the collection and transport of specimens on the recovery of bacteria from peritonsillar abscesses. *J Clin Microbiol*, 2: 504-509.

Indresano, A.T, Haug, R.H. and Hoffman, M.J., 1992. The third molar as a cause of deep space infections. *J Oral Maxillofac Surg*, 50: 868.

Iruka N. Okeke, Anibal Sosa. Antibiotic Resistance in Africa discerning the enemy and plotting a defense; www.tufts.edu/med/apua/Pubs/Articles/**africa**health.pdf, 2003.

UNIVERSITY of the
WESTERN CAPE

Jousimies-Somer, H.R., Summanen, P.H., Finegold, S.M., 1999. Bacteriodes, Porphyromonas, Prevotella, Fusobacterium and other anaerobic gram-negative rods and cocci. *In:* P.R. Murray, E.J. Baron and M.A. Pfaller, eds. *Manual of Clinical Microbiology*. 7th Edition. Washington, D.C. ASM Press, 690 – 711.

Kannangara, D.W., Thadepalli, H. and McQuirter, J.L., 1980. Bacteriology and treatment of dental infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 50: 103.

Kasten, M.J., 1999. Clindamycin, metronidazole, and chloramphenicol. *Mayo Clin Pro*, 74: 825–833.

Key HIV Statistics. 2008. [online] Available: http:// www.tac.org.za.

Khemaleelakul, S., Baumgartner, J.C. and Pruksakorn, S., 2002. Identification of bacteria in acute endodontic infections and their antimicrobial susceptability. *Oral Surgery Oral Pathology Oral Radiology Endod*, 94: 746–755.

Killey, H.C., Seward, G.R and Kay, L.W., 1975. *An outline of oral surgery*, Part I. Revised reprint, Bristol: J Wright and Sons Ltd. 74–123, 148–156.

Kirkland, K.B., Briggs, J.P. and Trivette, S.L., 1999. The impact of surgical-site infections in the 1990's: attributable mortalily, excess length of hospitalisation and extra costs. *Infect Control Hosp Epidemiol*, 20: 725–30.

Klimek, J., Marsik, F. and Bartlett, R., 1976. Clinical, epidemiologic and bacteriologic observations of an outbreak of methicillin-resistant stapyhlococcocus aureus at a large community hospital. *Am J Med*, 61: 340-345.

Koneman, E.W., Allen, S.D. and Dowell, V.R., 1988. *Color atlas and textbook of diagnostic microbiology*. 3rd edition. Philadelphia: Lippincott, 17–18.

Krishnan, V., Johnson, J.V. and Helfrick, J.F., 1993. Management of Maxillo-Facial infections: A review of 50 cases, *J Oral Maxillofac Surg,* 51: 868.

Krogh, H.W., 1951. Extraction of Teeth on the presence of acute infections. *Journal of Oral Surg,* 9: 136-151.

Krucke, G.W., Grimes, D.E., Grimes, R.M. and Dang, T.D., 2008. Antibiotic resistance in staphyloccocus aureus–containing cutaneous abscesses of patients with HIV. *Am J Med,* 27 (3): 344 – 347.

Kumar, P. and Clarke, M., 1998. *Clinical Medicine*. *In*: P. Kumar, M. Clark, eds. W.B. Saunders. London, 23-71.

UNIVERSITY of the WESTERN CAPE

Kuriyama,T., Karasawa, T., Nakagawa, K., Saiki, K., Yamamoto, E. and Nakamura, S., 2000. Bacteriologic features and antimicrobial susceptibility in isolates of oro-facial odontogenic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 90: 600-608.

Kuriyama,T., Karasawa, T., Nakayawa, K., Yamamoto, E. and Nakamura, S., 2002. Bacteriology and antimicrobial susceptibility of grown positive cocci isolate from pus specimens of orofacial odontogenic infections. *Oral Microbiol Immunol*, 17: 132-135.

Langford, F.J.P., Moon, R.E. and Stolp, B.W., 1995. Treatment of cervical necrotising fasciitis with hyperbaric oxygen therapy. *Otolaryngol Head Neck Surg*, 112: 274- 278.

Levitt, G.W, 1976. Cervical fascia and deep neck infections. O*rolaryngolocic Clinics of North America,* 9 (3): 703-715.

Lewis, M.A., McFarlane, T.W. and McGowan, D.A., 1990. A microbiological and clinical review of the acute dental abscess. *Br J Oral Maxillofac Surg*, 28: 359–366.

Madhi, S.A., Petersen, K., Madhi, A., Khoosal, A., Klugman, K.P., 2000. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired and lower respiratory tract infections in human immunodeficiency virus type-1 infected children. *Clin infect Dis*, 3: 170-176.

Marino, P.L., 2007. *The ICU Book*. 3rd Edition. Philadelphia. Lippincott Williams & Williams. 211–255, 697–913.

McIntosh, N., 2002. Intensive care monitoring; past, present and future. *Clin Med,* 2: 349-355.

Miller, M., Haddad, A.J., 1998. Cervicofacial actinomycosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 85: 496 – 508.

Miloro, M., Ghali, G.E., Larsen, P.E., Waite, P.D. 2004. *Peterson's Principles of Oral and Maxillofacial Surgery*. 2nd Ed. Hamilton, London. B.C. Decker Inc. 277-293.

Mindel, A. and Tenant-Flowers, M., 2001. ABC of AIDS: Natural History and Management of early HIV infection. *Br Med J,* 322 (7297): 1290-1293.

Mangundjaja, S. and Hardjawinata, K., 1990. Clindamycin versus ampicillin in the treatment of odontogenic infections. *Clin Ther*, 12: 242–249.

Morey, G.F., Moule, E.J. and Higgins, T.J., 1984. Pyogenic dental infections, a restrospective analysis. *Australian Dental Journal,* 29 (1): 150-153.

Neville, B.W., Damm, D., Allen, C.M., Bouquot and J.E., 2002. *Oral and Maxillofacial Pathology*. Philadelphia. Saunders. 137-308.

UNIVERSITY of the
WESTERN CAPE

Nuss, R.C. and Cunningham, M.J., 1993. Paediatric head and neck masses, cysts, sinuses and tumours. *Curr Op otolaryngol. Head Neck Surg*, 1: 153–158.

Okeke, I.N. and Sosa, A., 2003. *Antibiotic Resistance in Africa discerning the enemy and plotting a defense* [online]. Available from: www.tufts.edu/med/apua/Pubs/Articles/africahealth.pdf. [Accessed 9 April 2009].

Panlilo, A.L., Cluver, D.H. and Gaynes, R.P., 1992. Methicillin-resistant Staphylococcocus aureus in US hospitals 1975-1991. *Infect Control Hosp Epidemiol*, 13: 582-586.

Peters, E.S., Fong, B. and Wormuth, D.W., 1996. Risk factors affecting hospital length of stay in patients with odontogenic infections. *J Oral Maxillofac Surg*, 54: 1386.

Peterson, L.J., Ellis, E., Hupp, J.R., Tucker, M.R., 2004. Principles of management and prevention of odontogenic infections. *In*: L.J. Peterson, E. Ellis, J.R. Hupp and M.R. Tucker, eds. *Contemporary oral and maxillofacial surgery*. 4th Ed. St. Louis (MO): Mosby, 120-374.

Piecuch, J.F., 1962. Odontogenic infections. *Dental Clinics of North America*, 26 (1): 129-145.

Pryor, J.P., Reilly, P.M. and Shapiro, M.B., 2000. Surgical airway management in the intensive care unit. *Crit Care Clin*, 16: 473–488.

Rega, A.J., Shahid, R.A. and Ziccordi, V.B., 2006. Microbiology and antibiotic sensitivities of head and neck space infections of odontogenic origin. *J Oral Maxillofac Surg*, 64: 1377-1380.

Robertson, D. and Smith, A.J., 2009. The microbiology of acute dental abscess. *J Med Microbiol,* 58:155–162.

Sakamoto, H., Kato, M., Sato, T. and Sasaki, J., 1998. Semi-quantative bacteriology of closed odontogenic abscess. *Bull Tokyo Dent Coll*, 39: 103–107.

Schuster, G.S., 2002. Microbiology of the oro-facial region *In*: R.G. Topazian, M.H. Goldberg and J.R. Hupp, eds. *Oral and maxillofacial infections*. 4th Edition. Philadelphia: WB Saunders Company, 30–42.

Shafer, W.G., Hine, M.K. and Levy, B.M., 1983. *A textbook of oral pathology*. 4th Edition. Philadelphia, London, Toronto: WB Saunders Company, 511 – 527.

Siqueira, J.F. and Rocus, I.N., 2004. Treponema species associated with abscess of endodontic origin. *Oral Microbiol Immunol*, 19: 336-339.

Siqueira, J.F., Rocas, I.N., Oliveira, J.C. and Santos, K.R., 2001. Detection of putative oral pathogens in acute periradicular abscesseses by 16s rDNA–directed polymerase chain reaction. *J endod*, 27: 164–167.

UNIVERSITY of the WESTERN CAPE

Skitarelić, N., Mlandinu, R., Morovic, M. and Skitarelić, N., 2003. Cervical necrotising fasciitis: Sources and outcomes. *Infection*, 31 (1): 39–44.

Sloand, E.M., Klein, H.G. and Banks, S.M., 1992. Epidemiology of thrombocytopenia in HIV infection. *Eur J Haematol*, 48: 168.

Smith, A.J., Jackson, M.S. and Bagg, J., 2001. The ecology of staphylococcal species in the oral cavity. *J Med Micro*, 50: 940-946.

South Africa Country Progress Report 2008, 2008 [online]. Available from: http://data.unaids.org/pub/report/2008/south_africa_2008_country_progress_report_en.pdf. [Accessed 25 February 2009].

Stefanopoulos, P.K. and Tarantzopoulou, A.D., 2005. Facial bite wounds: management update. *Int J Oral Maxillofac Surg*, 34: 464-72.

Storoe, W., Haugh, R.H. and Lillich, T.T., 2001. The changing face of odontogenic infections. *J Oral Maxillofac Surg*, 59: 739.

Talan, D.A., Citron, D.M., Abrahamian, F.M., Moran, G.J., Goldstein, E.J.C., 1999. Bacteriologic analysis of infected dog and cat bites. *N Engl J Med,* 340: 85-92.

Tan, P.T., Chang, L.X., Huan, Y.C., Chiu, C.H., Wang, C.R. and Lin, Y.T., 2001. Deep neck infections in children. *J Microbiol Immunol Infect*, 34: 287-292.

The South African Department of Health Study 2006 [online]. Available from: http://www.avert.org/aidssouthafrica.htm [Accessed 2 April 2009].

Treatment Action Campaign. *Key HIV Statistics*, 2008 [online]. Available from: http://www.tac.org.za. [Accessed 4 April 2009].

Turner-Thomas, T., 1908. Ludwig's Angina. An anatomical, clinical and statistical study [Medline]. *Ann Swg*, 47: 161–163. [Accessed 2 April 2009].

UNAIDS, 2008. *Sub-saharan Africa Aids epidemic Update. Regional Summary. World Health Organization, Geneva* [online]. Available from: http://unaids.org/pub/report/2008/ sub_saharan_africa_aids_epidemic_update. [Accessed 2 April 2009].

Williams, A.C. and Guralnick, W.C., 1943. The diagnosis and treatment of Ludwig's angina: a report of twenty cases. *N Engl J Med,* 228: 443.

Williams, A.C., 1940 Ludwig's angina. *Surg Gynecol Obstet*, 70: 140.

Winther, B., Vickery, C.L. and Gross, C.W., 1998. Microbiology of the maxillary sinus in adults with chronic sinus disease. *Am J Med Sci*, 316: 13–20.

UNIVERSITY of the
WESTERN CAPE

Woods, R., 1978. Pyogenic dental infections: A ten year review. *Australian Dental Journal,* 23: 107–111.

World Health Organization, 2004. *The world health report – changing history* [online]. Available from: http:// www.int/entity/who/2004/annex/topic/enannex 2 en pdf. [Accessed 4 April 2009].

Young, D.M**.,** 2004. An epidemic of methicillin-resistant Staphylococcus aureus soft tissue infections among medically underserved patients**.** *Arch Surg*, 139: 947-951.

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APPENDIX I: DATA CAPTURE SHEET

Data Capture Sheet	**Study number:**
	Yes/ has it: √
Patient Sticker/ Write (incl DOB)	
Previous medical History: Retro + ☐	Retro - ☐ Uknown - ☐
Diabetes ☐	Auto immune disease ☐ Specify ……
Previous Surgical History:	
Allergies:	
Social History:	
Examination: L – left R - Right	
Abscess: Buccal Space	Sublingual Space
Submandibular	Mental Space
Lat Pharyngeal	Parotid Space
Canine Space	Parapharyngeal
Superficial Temporal	Deep Temporal
Ludwigs angina	Mediastinitus
Cavernous Sinus Thrombosis	
Other Specify:…….	
Possible Causes: Skin ☐	Teeth (specify) ☐ …….
Other…….	
Other Forms of infection (Osteomyelitis, Necrotising Fasciitis….)	
Specify (Details)…….	

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Other notes:
Plan: Retro test result ☐ ☐ No Consent Given ☐
MCS Result & FBC & U&E:
Number of Redebridements:

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APPENDIX II: STATISTICS CAPTURE FORM

Statistics capture form

X : No √ : Yes

Study number:						
Age			**Sex**			
Previous medical History: HIV+		HIV -		Uknown -		
Diabetes		Auto immune disease		pecify ……		
Allergies:…….						
Location:	**Left**	**Right**		**Left**	**Right**	
Abscess: Buccal Space			Sublingual Space			
Submandibular			Parotid Space			
Lat Pharyngeal			Parapharyngeal			
Canine Space			Deep Temporal			
Superficial Temporal			Submental Space			
Ludwigs Angina						
Other Specify:…….						
Possible Causes: Skin		Teeth (specify)		…….		
Septic Fracture		Unknown				
Other…….						
Other Forms of infection: (Osteomyelitis, Necrotising Fasciitis….)						
Other Specify:…….						
HIV: Positive:		CD4 count:		Negative:		
MCS Results: Mixed growth:		Normal Flora:		No Growth:		
Was antibiotics for No Growth	Yes		No	Types…….		
Gram Stain: Gram Positive cocci						
Gram Positive bacilli						
Gram Negative cocci						
Gram Negative bacilli						

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Microbiology and Antibiotic Resistance:

Organisim	Penicillin	Cloxacillin	Gentamycin	Ciprofloxacin	Erythromycin	Clindamycin	Vancomycin	Clotrimoxazole	Other
Staph aureus									
Strep constellatus									
Klebs pneumonia									
Strep Thoraltensis									
Strep salivarius									
Strep sanguinis									
Strep intermedius									
Strep gordonii									
Strep anginosus									
Alpha haemolytic Strep									
Beta haemolytic Strep (GF)									
Beta haemolytic Strep (C)									
Strep parasangauinis (Strep viridans)									
Strep pyogenes									
E coli									
Enterobact cloacae									
Beta haemolytic Strep (GG)									
Other									

S – Susceptible R – Resistant I - Intermedidiate

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Abnormality in Blood Tests:

Blood Parameter	Blood Value
White Cell Count	
Haemoglobin	
Platelets	
Sodium	
Potassium	
Chloride	
Urea	
Creatinine	

Number of redebridements/re- incision and drainage: ☐

Were antibiotics changed during treatment? Yes: ☐ No: ☐

Specify Change……

If patient admitted to hospital, indicate length of stay? ☐☐

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APPENDIX III: MICROBIOLOGY STANDARD OPERATING PROCEDURES

PROCEDURE FOR PROCESSING OF PUS SWABS AND PUS SPECIMENS

Any copy without original signature in red is an UNAUTHORIZED document

PURPOSE

To provide a uniform procedure for the culture of pus swabs and pus specimens

INTRODUCTION

The accumulation of pus, either within an abscess or exuding from a sinus tract or from a mucocutaneous surface, is one of the vital indicators of local sepsis. Varying degrees of redness, pain, and swelling may also be present. Exogenous wound infections include those associated with traumatic injury - wounds or decubitus ulcers, animal or human bites, burns, or foreign bodies in the skin or mucous membranes.

Endogenous wounds and abscesses may be associated with appendicitis, cholecystitis, empyema, septic arthritis, sinusitis, cellulites, osteitis or many other internal infections. Many of these infections are derived secondary to invasive procedures, surgical manipulations, or placement of prostheses. Others derive from haematogenous spread from other primary sites of infection or by direct extension of bacterial from ruptured viscera, particularly the large intestine. Effective treatment of wound infection usually includes drainage and removal of foreign bodies. Suction irrigation may be helpful in resolving wound infections.

Species commonly recovered from wounds include *Escherichia coli*, *Proteus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Enterobacter* sp, enterococci, other streptococci, *Bacteroides* sp, *Prevotella* sp, *Clostridium* sp, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (coagulase-negative Staphylococcus). Care should be taken to ensure that aerosol formation is kept to a minimum during the processing of these specimens.

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RESPONSIBILITY

All competent technical staff may process these specimens. Students may process specimens under supervision of qualified technologists or technicians.

SPECIMEN

Specimens received on swabs are more likely to contain colonizing organisms and normal skin micro-organisms than specimens obtained by aspiration with needle and syringe after appropriate surface decontamination.

Pus swabs from:
burns
wound
eye
skin
gall bladder
lung, heart (post mortems)
ANA swabs

Pus from the following organs:
Brain
Liver

Pus with granules
- cover for *Actinomyces* (anaerobic culture)

EQUIPMENT AND MATERIALS

Appropriate culture media
Bacteriological loop

CO_2 incubator

Anaerobic cabinet

Microscope slides
Light microscope

PREPARATION AND STORAGE OF MEDIA

All culture plates are stored at 2-8°C or on bench before use.

METHOD

Microscopic examination:
Prepare slides for microscopy ensuring that the material is thinly and evenly spread on the slide surface by using the tip of the swab and rolling the swab. Care must be taken to prevent extraneous contamination of the specimen. Perform Gram stain (see Gram stain SOP)

Pus from brain; liver and lung are VERY important specimens. A Gram Stain must be done immediately and must be reported to the registrar or pathologist. N.B. remember to make slides with thin layer of fluid to prevent wash off when stain is made. Heat slides before inoculation to remove waxy layer on slide. Inoculate using swab or pipette rather than loop. Methylene blue stain may be helpful in differentiating cells.

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If culture for TB or ZN is requested, the specimen is referred to the TB lab

If fungal culture is requested, process according to Mycology SOP.

Inoculation of pus swabs and pus onto appropriate media (Date all plates!):
Blood agar (BA) (CO_2/18 - 24h) at 35ºC

McConkey agar (McC) (CO_2/18 - 24h) at 35ºC

For pus, incubate plates for 18-48h
Anaerobic culture for following:
pus
swabs in anaerobic transport media
swabs from brain abscesses or deep wounds
swabs from human/animal bite wounds
Inoculate Thioglycollate broth or CMM, Nalidixic acid agar and AAM (date all plates)
A metronidazole (Flagyl) disc is also placed on the nalidixic plate and the AAM plate at the junction of the heavy inoculum
Cooked Blood Agar (CBA)(CO_2/18 - 48h) at 35 ºC

For pus specimens and if diagnosis is one of the following, add a Cooked Blood Agar plate incubated for 18-48h at 35 ºC:
septic arthritis
osteitis
septic abortion, miscarriage etc.
face-skin swabs in children (< 5 yr)
All post mortem swabs
Eye swabs
PID= Pelvic Inflammatory Disease
ASO =Acute salpingo-ophalitis

Any specimen with a diagnosis where a growth of a *Haemophilus* species, *N. gonorrhoeae* or *N. meningitidis* can be expected, add a CBA plate. Incubate CBA CO_2 for 24 - 48h.

All swabs from the liver/gall bladder area: Add a XLD plate. Incubate aerobically for 18-24h.

Inoculation of Eye swabs onto appropriate media:
BA/CBA (CO_2/18 - 48h) 35ºC
McConkey agar (CO_2/18 - 48h) 35ºC

On occasion we receive a BA and CBA that have been directly inoculated in the ward. Streak these plates again for single colonies. Incubate plates 18 - 48h CO_2 at 35ºC

Eye fluid:
Inoculate as above+ wet preparation to look for Acantamoeba and Echinococcus.
(Acantamoeba: inoculate on a "lawn" of E. coli on Page's saline agar medium – only on request)

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Interpretation of results

Clearly and accurately record all observations, smears, test results, and actions taken at each step of the workup.

After 24 hour incubation:
Examine the aerobic plates for evidence of growth.
Identify all distinct colony types, and identify them according to defined laboratory protocol (see identification SOPs).
Perform antimicrobial susceptibility testing on organisms from well-isolated colonies.
All microscopic and aerobic culture results are recorded on Disalab and authorized with anaerobic culture results to follow.

Anaerobic incubation:
Follow anaerobic plates up for 5 days before reporting as no growth on worksheet. Identify all anaerobic organisms according to anaerobic SOPs and report on disalab

Interpretation of culture media and the identification of probable pathogens
The extent of testing depends on several factors, such as specimen source collection method (swab versus aspiration), and results of direct Gram stain

Perform definitive identification and antimicrobial susceptibility testing on the following:

Any quantity of probable pathogens, e.g. *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes*

Perform limited identification on the following:
Probable skin "contaminants," which include coagulase-negative staphylococci, diphtheroids, viridans streptococci, and *Bacillus* spp., with many squamous epithelial cells and/or few to no pus cells on original smear

Do not perform identification or antimicrobial susceptibility testing on the following:
More than three species of intestinal flora from sites such as in intra pelvic area, decubitus ulcer, perianal abscess or fistula, and intestinal drainage

Provide a descriptive identification of the organisms present.

If one organism is predominant it should be identified and antimicrobial susceptibility testing should be performed.

LIMITATION OF METHOD

See SOP on uncertainty of measurement

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REFERENCES

American Microbiology Association Manual: ISENBERG 1998.

I, THE UNDERSIGNED, STATE THAT I HAVE READ AND UNDERSTOOD THE CONTENTS OF THIS SOP.

DATE	NAME	SIGNATURE

SWABS

BA 35°C CO_2
McC 35°C CO_2
Gram stain

For anaerobic culture add:
Thioglycollate broth/CMM 35°C Aerobic
AAM (ANA) 35°C ANA + Mz disc
Nalidixic Acid (ANA) 35°C ANA + Mz disc

Add:
SS/XLD 35°C Aerobic

EYE SWABS AND EYE FLUIDS

BA/Cooked BA 35°C CO_2
McC 35°C CO_2
Gram stain

Corneal scrape plates – streak for single colonies

Eye fluids:
Inoculate as above + anaerobic culture + WET PREP for Acanthamoeba and Echinococcus.

If no growth or very fine growth after 24hrs, incubate a further 24 hrs

FLUIDS/ASPIRATES
[PD Fluid Only Gram]
BA/Cooked BA 35°C CO_2
McC 35°C CO_2
Thioglycollate broth/CMM 35°C Aerobic
AAM (ANA) 35°C ANA + Mz disc
Nalidixic Acid (ANA) 35°C ANA + Mz disc

Gram stain
Methylene Blue stain (Pleural Fluids)

Liver aspirates: Wet Prep to look for *Entamoeba histolytica* and *Echinococcus granulosus.*
For semen, add New York City medium, 35°C CO_2

If no growth or very fine growth after 24hrs, incubate a further 24 hrs

POST MORTEM SWABS

BA/Cooked BA 35°C CO_2
McC 35°C CO_2

No Gram

If no growth or very fine growth after 24hrs, incubate a further 24 hrs

PUS

BA/Cooked BA 35°C CO_2
McC 35°C CO_2
Thioglycollate broth/CMM 35°C
Aerobic
AAM (ANA)
35°C
ANA + Mz disc
Nalidixic Acid (ANA) 35°C ANA + Mz disc

Gram stain
(Methylene Blue stain)

If no growth or very fine growth after 24hrs, incubate a further 24 hrs

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TISSUE

Work in **LAMINAR FLOW CABINET!**
BA/Cooked BA 35°C CO_2
McC 35°C CO_2
Thioglycollate broth/CMM 35°C
Aerobic
AAM (ANA)
35°C
ANA + Mz disc
Nalidixic Acid (ANA) 35°C ANA + Mz disc
Gram stain

If no growth or very fine growth after 24hrs, incubate a further 24 hrs

GASTRIC BIOPSIES FOR H. PYLORI CULTURE

BA 35-37°C CO_2 5-7days

CATHETER TIPS/ CVP/ Provox

Add Thioglycollate or Robertson's Meat Broth (CMM) to specimen, shake well and use standard 4mm loop to inoculate plates. Use glass rods to streak for single colonies.

BA 35°C CO_2
McC 35°C CO_2
No gram stain

If no growth or very fine growth after 24hrs, incubate a further 24 hrs

IUD

Add Robertson's meat broth (CMM) or thioglycollate broth and vortex
Inoculate Nalidixic acid plate to screen for Actinomyces (anaerobic) x14days
Gram stain
Incubate broth aerobically at 35°C x 14 days, inspect daily and subculture when turbid

HEART VALVES

USE STERILE GLOVES to prevent heart valve contamination.

Process in Laminar Flow Cabinet!

Place valve in CMM (cooked meat broth/Robertson's meat broth) and incubate overnight.
No Gram stain
Subculture next day:
CBA (CO_2/18-48h) 35ºC, 48hrs
AAM(anaerobically) 35ºC, 48hrs – 5 days
CMM 35ºC Inspect daily: If turbid make gram stain and inoculate appropriate plates.
If clear, subculture again on day 14.

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NB!!! Add a NEW YORK CITY plate to ALL specimens where gram negative diplococci are seen in the gram!

PROCEDURE FOR PROCESSING OF FLUIDS

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PURPOSE

To provide a uniform procedure for the microscopy and culture of sterile body fluids specimens

INTRODUCTION

Infection of normally sterile body fluids often results in severe morbidity and mortality; therefore, rapid and accurate microbiological assessment of these samples is important to successful patient management. Most organisms infecting these sites are not difficult to culture, but finding a low number of commensal organisms does present an interpretative challenge. Any microorganism found where no resident microflora is present must be considered significant. With the increased use of prostheses, immunosuppressive therapeutic regimens, and long-term care of individuals with chronic debilitating disease, the likelihood of true infection with this type of commensal organism has increased. The final interpretation can be made by the pathologist in consultation with the clinician on the basis of the clinical status of the patient.

RESPONSIBILITY

All competent technical staff may process these specimens. Students may process specimens under supervision of qualified technologists or technicians.

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SPECIMEN

Fluids (usually sterile):
pleural fluid
pericardial fluid
peritonial fluid (ascites fluid)
sinovial fluid
eye fluid
aspirates (liver; lung etc.)
semen

EQUIPMENT AND MATERIALS

Appropriate culture media
Sterile cotton swabs
Bacteriological loop
CO2 incubator
Anaerobic cabinet
Microscope slides and coverslips
Light Microscope
Sterile test tubes
Centrifuge
Sterile Pasteur Pipettes
Rubber teat

PREPARATION AND STORAGE OF MEDIA

All culture plates are stored at 2-8°C or on bench before use.

PROCEDURE

Centrifuge all fluids that are not full of pus or blood for 10 minutes at ±2000 r.p.m.
Remove supernatant using pipette into another empty sterile tube.
Use the sediment to inoculate all media and slides for wet preparation and Gram Stain. All Pleural fluids get a Gram Stain and a Methylene blue stain.
N.B. remember to make slides with thin layer of fluid to prevent wash off when stain is made.
Heat slides before inoculation to remove waxy layer on slide.
Inoculate using swab or pipette rather than loop.
If culture for TB or ZN is requested, the specimen is referred to the TB lab

Wet preparation
A wet preparation is performed on all liver and lung aspirates and eye fluids to detect parasites, as well as on all fluids where parasites requested. Synovial fluid is examined for uric acid crystals where requested.

Using a sterile pipette, place a drop of neat fluid onto a glass slide. Coverslip and read microscopically using dry lens (40X or 10X).

Inoculation of pleural/sinovial/pericardial, peritoneal (ascites) and semen onto appropriate media:
Using a sterile pipette, gently mix the sediment and by aspiration, transfer the sample to be plated.
Drop fluid onto one quadrant of each plate medium and the remainder into the broth.

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Culture media (Date all plates!):

BA/CBA (CO_2/18 - 48h)	35ºC
McConkey agar (CO_2/18 - 48h)	35ºC
Nalidixic Agar with metronidazole (anaerobically/ 5 days)	35ºC
Ameliorated anaerobic media with metronidazole (anaerobically/ 5 days)	35ºC
Thioglycollate broth or CMM (aerobic)	±35ºC

For **semen**, add New York City medium

ALL CELL COUNTS) ON SPECIMENS OTHER THAN CSF ARE DONE BY HAEMATOLOGY. (IF NECESSARY MAKE COPIES OF FORM AND SPLIT SAMPLE)AND DOCUMENT

Aspirates:
Liver and lung aspirates: Make a wet preparationto look for *Entamoeba histolytica* and for *Echinococcus granulosus.*
Other: e.g. aspirate from a fistula, aspirate from a wound. Process as for fluids.

Eye fluid:
Process as fluids

Wet preparation to look for *Acantamoeba* and *Echinococcus.*
Acantamoeba: inoculate on a "lawn" of *E. coli* – (only on request -special media required.)

Interpretation of results:
Clearly and accurately record all observations, smears, test results, and actions taken at each step of the workup.

After 24 hour incubation:
Examine the aerobic plates and the broth for evidence of growth.
Identify all distinct colony types, and identify them according to defined laboratory protocol (see identification SOPs).
Perform antimicrobial susceptibility testing on organisms from well-isolated colonies.
Re-incubate blood agar and chocolate agar and broth media found to have no growth or very scanty growth after 24 hours.
If primary culture media yield no growth after 24 hrs, but organisms seen on original Gram stain or broth turbid, do a Gram stain and subculture accordingly (see SOP on subcultures)

After 48 hour incubation:
Examine the primary plates, including anaerobic plates and subculture plates.
If growth on plates, identify any additional organisms found on the primary or subculture media, and perform antimicrobial susceptibility testing.

LIMITATIONS OF THE METHOD

See SOP on uncertainty of measurement

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QUALITY CONTROL

See Internal Quality Control SOP.

REFERENCES

American Microbiology Association Manual: ISENBERG 1998.

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DATE	NAME	SIGNATURE

PROTOCOL FOR PRIMARY IDENTIFICATION OF ANAEROBES

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INTRODUCTION

Most deep-seated abscesses and necrotizing lesions involving anaerobes are polymicrobial and may include obligate aerobes, facultative anaerobes, or microaerophiles as concomitant microorganisms. These microorganisms, acting in concert with trauma, vascular stasis or tissue necrosis, lower the oxygen tension and the oxidation-reduction potential in tissues, and provide favourable conditions for obligate anaerobes to multiply.

The more common endogenous anaerobic infections are listed below:

- Abscess of any organ
- Actinomycosis
- Antibiotic-associated diarrhoea and colitis
- Aspiration pneumonia
- Complications of appendicitis or cholecystitis
- Crepitant and noncrepitant cellulitis
- Clostridial myonecrosis
- Dental and periodontal infections
- Endocarditis
- Meningitis, usually after brain abscess
- Necrotizing pneumonia
- Osteomyelitis
- Otitis media
- Peritonitis
- Septic arthritis
- Sinusitis
- Subdural empyema
- Tetanus
- Thoracic empyema

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It is essential to isolate and identify anaerobic bacteria because:-

- these infections are associated with high morbidity and mortality, and
- the treatment of the infection varies with the bacterial species involved.

Isolation of anaerobes requires more exacting conditions than subsequent culture of the pure isolates. Therefore optimum conditions should be used for isolation. Once pure cultures are obtained exacting conditions are not nearly so important, but they will allow more rapid and more definite characterization.

RESPONSIBILITY

All technicians and medical technologists who are trained to perform these test procedures are responsible.

SPECIMEN TYPE

pus

swabs in anaerobic transport media

swabs from brain abscesses or deep wounds

swabs from human/animal bite wounds

fluids

tissue specimens

IUD (for Actinomyces)

EQUIPMENT

Anaerobic jar
Gas cylinders
Anaerobic chamber
Slides
Loops

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MEDIA

For primary isolation of anaerobes:
non-selective media AAM (appropriate anaerobic media). AAM is a Brucella agar supplemented with hemin, vitamin K1 and 5% laked sheep blood. It is recommended by the CLSI as it supports good growth of (essentially) all anaerobes.
selective media (Naladixic Agar)
a broth media CMM (cooked meat media) or thioglycollate broth (provide backup if anaerobic chamber fails, for enrichment for small numbers or when growth is inhibited on primary culture media)
Metronidazole disc

For further identification:
anaerobic Egg-yolk agar
Mastring
Anaerobic BBL crystal

REAGENTS

Gram stain reagents
chromogenic nitrocephin
Clostridium perfringens anti-toxin

PREPARATION AND STORAGE OF MEDIA
All culture plates are stored at 2-8°C or on bench before use.

PROCEDURE

PROCESSING SPECIMENS:
Visual examination: look for and take note of:
blood
purulence
necrotic tissue
foul odour
sulphur granules

Specimen preparation:
Vortex grossly purulent fluids in transport vial to ensure even distribution of micro-organisms.
Grind bone or tissue. Robertson's meat broth or thioglycollate broth may be added to make thick paste.
Wring out swabs in liquid medium (e.g. Robertson's meat broth or thioglycollate broth) and then treat as a liquid specimen.
Centrifuge large volumes of non-purulent material and use the sediment to work with.

Inoculation of media:
aerobic media as appropriate for type of specimen
anaerobic media , incubate in anaerobic chamber for 48hrs. Additional periods of incubation may be necessary to recover some anaerobes
thioglycollate broth/CMM, ±35°C aerobically
Slide for Gram stain

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GRAM STAIN:
Anaerobes can be Gram variable:

always try to use a young culture if possible
airdry and fix with methanol to preserve all morphology

INTERPRETATION OF GRAM STAINS:

large Gram positive rods with boxcar-shaped cells and no spores usually indicate *C. perfringens*
Gram negative coccobacillary forms suggest pigmenting *Bacteroids* or *Porphyromonas* group.

Thin Gram negative bacilli with tapered ends suggest *Fusobacterim nucleatum* or *Capnocytophaga sp.*

Pleomorphic pale staining Gram negative bacillary suggest *Bacteroides spp.*

Very small Gram negative cocci suggest *Veillonella spp.*

Long, thin branching Gram positive bacilli=? *Actinomyces or Norcardia.* A modified kinyoun acid fast stain should be done to confirm this.

AFTER 48 HOURS:

Perform initial plate examination.

Record: colony type, noting pitting, swarming, hemolysis or "greening" of medium. (see Table 1)

Select isolated colonies and subculture on Blood Agar or Chocolate Agar for aerotolerance testing (aerobic incubation) and a fresh Naladixic acid agar + metronidazole disc (anaerobic incubation) to ensure organism is a strict anaerobe

If no growth after 48hrs, re-incubate.

If no growth on primary plates on Day 5, subculture broth onto anaerobic media and incubate for 48hrs

.

Table 1

COLONY MORPHOLOGY	**POSSIBLE IDENTIFICATION**	**SUPPLEMENTAL MEDIUM**
Agar pitting	*Bacteroides ureolyticus* Group	
Black pigmentation	*Porphyromonas & Prevotella spp*	*EYA for lipase (*Prevotella intermedia)*
Brick red fluorescence	*Prevotella intermedia Prevotella loeschii*	EYA for lipase
Chartreuse fluorescence	*Fusobacterium*	EYA for lipase
Double-zone beta hemolysis	*Clostridium perfringens*	EYA for lecithinase
"Fried egg"	*Fusobacterium necrophorum*	EYA for lipase,
"Greening" of medium	*Fusobacterium spp*	EYA for lipase
Large with irregular margin	*Clostridium spp*	EYA for lecithinase
"Medusa-head"	*Clostridium septicum*	
"Molar tooth"	*Actinomyces spp*	
Speckled	*Fusobacterium nucleatum*	
Swarming growth	*Clostridium septicum Clostridium sordelii*	

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* EYA = egg yolk agar

AFTER another 48 HOURS:

If pure growth on Nalidixic plate, Gram stain to determine identification procedure to be followed.

IDENTIFICATION OF ANAEROBES

MAST ID SYSTEM MID 8 IDENTIFICATION MASTRING

The presumptive identification of anaerobes is done with the MID8 Identification Mastring method: Fenefold *et al* demonstrated the use of antibiotic susceptibility patterns to characterise Gram negative anaerobes, and Sutter and Finegold suggested a range of 6 high strength discs for this purpose. Sutter *et al* suggested the use of vancomycin to confirm Gram results and to eliminate *Clostridium spp*. The Mastring is placed on a Nalidixic plate inoculated with the anaerobe that is to be identified.

Strains are characterised according to the following table:

ORGANISM	**ANTIMICROBIAL**					
	Erythromycin	**Rifampicin**	**Colistin**	**Penicillin**	**Kanamycin**	**Vancomycin**
Gram negative bacilli						
Bacteroides fragillis group	*S*	*S*	*R*	*R*	*R*	*R*
Prevotella melaninogenica/oralis	*S*	*S*	*S**	*S**	*R*	*R*
Porphyromonas spp	*S*	*S*	*S*	*S**	*R**	*S**
Bacteroides ureolyticus	*S*	*V*	*S*	*S*	*S*	*R*
Fusobacterium mortiferum/varium	*R*	*R*	*S*	*S*	*S*	*R*
Other Fusobacterium spp	*R**	*R**	*S*	*S*	*S*	*R*
Gram negative cocci	S	S	S	S	S	R
Gram positive cocci	S	S	R	S*	V	S
Clostridium spp	S	S	R	S*	V	S
Gram positive bacilli (NSGPG)	S	S*	R	S*	V	S
Peptostreptococcus anaerobius	S	S	R	S	S/R	S

S = Sensitive

R = Resistant

R* = Majority resistant

S* = Majority sensitive

V = Variable

If necessary identify further using the anaerobic BBL commercial kit.

GRAM-NEGATIVE COCCI

This includes *Veillonella* species, *Acidaminococcus* species and *Megaspaera* species. *Veillonella* is the only frequently encountered species of clinical importance.

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Identification:

See Mastring reactions.

GRAM NEGATIVE BACILLI

Anaerobic gram-negative bacilli are the most commonly encountered anaerobes in clinical specimens, with *Bacteroides fragilis* isolated more frequently than any other anaerobes. The *B. Fragilis* group produces ÿ-lactamase and includes species resistant to many antibiotics commonly used to treat anaerobic infections.

This group includes *Bacteroides* species *Porphyromonas* species, *Prevotella* species and *Fusobacterium* species. These species are identified on the basis of colonial and cellular morphology, pigment production, susceptibility to antibiotic discs and certain rapid biochemical characteristics. Gram-negative anaerobic bacilli are the most commonly encountered anaerobes in clinical infections.

Pigmenting anaerobic gram-negative bacilli:

These may be divided into those that are saccharolytic i.e. *Prevotella* species and those that are asaccharolytic i.e. *Porphyromonas* species. Pigmentation is due to protoheme production.

Fusobacterium species

Fusobacterium are commonly involved in serious infections in various body sites and have been frequently isolated from blood cultures. *Fusobacterium necrophorum* is a very virulent anaerobe that may cause severe infection.

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Identification of Gram negative bacilli:

See Mastring reactions

GRAM POSITIVE COCCI

The anaerobic cocci are frequently encountered in the clinical laboratory in blood cultures and other body fluids and in a wide variety of wound and abscess specimens. Except for *Peptococcus niger* all former species of the genus *Peptococcus* were transferred to the genus *Peptostreptococcus*.

Identification:

See Mastring reactions

GRAM-POSITIVE BACILLI

Anaerobic gram-positive bacilli of human clinical relevance are divided into two distinct groups one genus of endospore formers (*Clostridium* species) and genera of non-sporeformers. *Clostridium* species can cause acute, severe or chronic infections. Some are highly pathogenic or toxigenic.

1. SPORE FORMING BACILLI

The anaerobic gram-positive, spore forming bacilli belong to the genus *Clostridium*.

Certain clostridia for example: *C tertium, C inulinum, C durum* and *C carnis* are aerotolerant and form colonies on 10% horse blood agar incubated in a 5% to 10% CO_2 (i.e. in microaerophilic conditions).

The following criteria are used to determine whether an isolate is an aero-tolerant *Clostridia* species or a facultatively anaerobic *Bacillus* species. Aerotolerant *Clostridia* will rarely form spores when grown aerobically and are catalase negative, whereas *Bacillus* species will rarely form spores when grown anaerobically and are catalase positive. To demonstrate spores, Gram-stained preparations are usually sufficient.

2. GRAM-POSITIVE NON-SPORE FORMING BACILLI

Included in this group are members of the following genera:

Actinomyces

Bifidobacterium

Lactobacillus

Mobiluncus

Propionibacterium

Eubacterium

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The nonsporeformers are infrequently significant and usually cause chronic disease. Many of these nonsporeformers are resistant metronidazole.

In cellular morphology members of these genera are usually pleomorphic gram-positive to gram-variable rods. If these organisms are observed on a direct gram smear of clinical material, cultures may require incubation for up to 2 weeks for the organisms to grow.

IDENTIFICATION

See Mastring reactions. If necessary, identify on anaerobic BBL crystal.

If Clostridium spp. according to Mastring results, put up egg- yolk agar with antitoxin (Nagler reaction) if available.

NAGLER REACTION

For this reaction use an egg-yolk medium, and smear one half of the surface with antitoxin (antitoxin serum made up of *C perfringens* and *C novyi*). The culture is then streaked in a single line across the plate at a right angle to the antitoxin.

The toxin lecithinase produces a precipitate (opalescence) around the growth in the line of streak in the absence of antitoxin, but is inhibited on the half of the plate with antitoxin. A positive control organism of

C perfringens should also be included in the test.

Cloudy (opaque) zone in medium around colony = lecithinase positive

Oily irridescent sheen over and immediately around growth = lipase positive.

An opaque zone may be present under the irridescent sheen.

C perfringens produces colonies showing double zones of haemolysis and the Nagler test is positive. A more recently described test, the reverse CAMP test is specific to *C perfringens* and reveals enhanced haemolysis of group B streptococci.

TEST ALL ANAEROBES FOR THE PRODUCTION OF -LACTAMASE

ÿ-lactamase testing using Nitrocefin

Chromogenic nitroceflin solution should be used. Add a drop of solution onto glass slide and smear inoculum directly onto spot. Should be read after 10 minutes.

Pink colour = positive

No colour = negative.

LIMITATION OF METHOD

See SOP on uncertainty of measurement

REFERENCES

1. American Microbiology Association Manual: ISENBERG 1998.

2. Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standards – Sixth Edition. M11-A6. 2004.

I, THE UNDERSIGNED, STATE THAT I HAVE READ AND UNDERSTOOD THE CONTENTS OF THIS SOP.

DATE	NAME	SIGNATURE

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