A 7-YEAR RETROSPECTIVE REVIEW OF THE MICROBIOLOGY OF DEEP NECK INFECTIONS IN ADULTS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

Sumaya Ahmed

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Otorhinolaryngology

Johannesburg, 2018.

PLAGIARISM DECLARATION

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I SUMAYA AHMED (Student number: 0202217J) am a student registered for the degree of Mmed ORL in the academic year 2018.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: S Ahmed Date: 29/1/2018

DEDICATION

- To my supportive and patient family
- To all the patients assisted by the ORL-HNS staff of CHBAH, locally and elsewhere
- To every aspiring colleague committed to the discipline of ORL-HNS (Otorhinolaryngology – Head & Neck Surgery)

ABSTRACT

This study is a seven year (01/07/08 - 30/06/15) retrospective review of the microbiology of deep neck infections in 52 adult patients at Chris Hani Baragwanath academic hospital. Micro-organisms isolated from patients with deep neck infections were analysed, including their antibiotic susceptibility patterns. The effectiveness of empiric usage of amoxicillin – clavulanic acid against commonly identified microbes and recommended alternative antibiotic usage were reviewed.The register records of 70 microscopy, culture, and antibiotic sensitivity results of specimens taken intraoperatively, in patients with deep neck infections who underwent surgical intervention, were analysed. Aerobic identified gram negative bacilli and streptococcus species; and anaerobic Prevotella, were the most frequently isolated microorganisms. Microbial sensitivity and resistance to amoxicillin – clavulanic acid was reported in 15% (n = 8) of patients with deep neck infections. Hence, the effectiveness of empiric usage of amoxicillin – clavulanic acid, against microbes commonly involved in deep neck infections in adults at Chris Hani Baragwanath academic hospital; cannot be proved nor disproved and is thus recommended as an option; alternative empiric antibiotic usage likewise cannot be recommended. Further periodic surveillance of microbial profiles and associated antimicrobial susceptibility results, in larger population samples of patients with deep neck infections; utilizing standardized protocols, is suggested.

ACKNOWLEDGEMENTS

Gratitude and appreciation are expressed to the following parties:

- Dr P. Pillay, my supervisor, who was always available for assistance and constructive suggestions.

- Mr Yusuf Ismail, for his guidance with regards to statistical analysis.

- Staff of the Department of ORL-HNS, University of the Witwatersrand.

- Staff of the National Health Laboratory Services (NHLS).

- Staff of the Department of Radiology at CHBAH.

TABLE OF CONTENTS

PLAGIARISM DECLARATION ii

DEDICATION iii

ABSTRACT iv

ACKNOWLEDGEMENTS v

LIST OF FIGURES viii

LIST OF TABLES ix

NOMENCLATURE xii

1 INTRODUCTION 1

1.1 Literature review 1

1.2 Hypothesis: 8

1.2.1 Study objectives 8

2. METHODOLOGY 9

2.1 Study Design 9

2.2 Study Population 9

2.3 Study Location 10

2.4 Study Period 10

2.5 Data Collection 10

2.6 Ethical Considerations 12

2.7 Problems and Potential Limitations 13

2.8 Data Analysis 13

3 RESULTS AND ASSOCIATED DISCUSSION 15

3.1 Analysis of the Study Population and Study Period 15

3.1.1 Analysis of age distribution of 52 patients 18

3.1.2 Analysis of gender distribution of 52 patients 19

3.1.3 Analysis of 70 specimen microbiological cultures in patients with DNIs....20
3.2 Analysis of Microbiological Cultures per Absolute Patient Count.....25
3.3 Antibiotic Sensitivity and Resistance Profiles of Microorganisms 36
3.3.1 Profiles of microbial susceptibility and resistance to amoxicillin – clavulanic acid.....48
3.3.1.1 Profile of microbial susceptibility to amoxicillin – clavulanic acid.....48
3.3.1.2 Profile of microbial resistance to amoxicillin – clavulanic acid.....51
3.4 Analysis of Histology Distribution.....61
3.4.1 Analysis of tissue necrosis distribution.....62
3.5 Microbiology of DNI and Pulmonary and Extra - Pulmonary Tuberculosis:.....63
Case Reports.....63
3.6 Fungal DNI.....72
3.7 Microbial MC&S and Related Morbidity and Mortality of Patients with DNIs....72
3.8 Sources of DNIs in Survivors versus Patients with DNIs who Demised.....85
4 SUMMARY, CONCLUSION AND RECOMMENDATIONS.....87
5 REFERENCES……94
6 APPENDICES……98
6.1 Appendix IA – Linked Secured File 1.....98
6.2 Appendix IB – Linked Secured File 2.....100
6.3 Appendix II – Ethics Clearance Certificate.....106
6.4 Appendix III – Supervisor’s Acknowledgement of Turn-it-in Report.....107

LIST OF FIGURES

Figure 3.1: Age distribution of 52 patients 19

Figure 3.2: Gender distribution of 52 patients.....20

Figure 3.3: Culture distribution of specimens 21

Figure 3.4: Age distribution per positive & negative cultures of patients 22

Figure 3.5: Gender distribution per positive & negative cultures of patients.....24

Figure 3.6: Comparative distribution of other gram positive and gram negative microorganisms isolated on microscopy only 35

Figure 3.7: Histology distribution: Categorisation of patients' specimen results 62

Figure 3.8: Tissue necrosis distribution: Categorisation of patients' specimen results 63

Figure 3.9: Sources of DNIs in Survivors 86

Figure 3.10: Sources of DNIs in patients who demised.....86

LIST OF TABLES

Table 3.1: 52 patient admissions over the 7 year study period (01/07/2008 – 30/06/2015)…….17

Table 3.2: Age statistics of all patients……..……18

Table 3.3: Gender distribution of all patients……19

Table 3.4: Categorization of microbiological culture results from 70 specimens of 52 patients with DNIs……..21

Table 3.5: Gender distribution per positive & negative cultures of patients……23

Table 3.6: Gender distribution per positive & negative cultures of patients: Categorisation of the results from 70 specimens…… 24

Table 3.7: Categorisation of microbiological culture results in 52 patients; per absolute patient count……...25

Table 3.8: Comparison of frequency of isolation of different microbiological categories of microorganisms in *35 patients with positive cultures; per absolute patient count……...26

Table 3.9: Number of different microorganisms identified per patient distribution, expressed as absolute numbers and percentages of the total study population with positive cultures…… 26

Table 3.10: All pathogens: 57 microorganisms identified on culture in 35 patients, expressed as absolute numbers and percentages of the total…….. 27

Table 3.11: 23 microorganisms identified on culture as single pathogens in 23 patients, expressed as absolute numbers and percentages of the total…….. 29

Table 3.12: 10 microorganisms identified on culture as co-existing pathogens in 5 patients, expressed as absolute numbers and percentages of the total…….. 30

Table 3.13: 12 microorganisms identified on culture as one of three pathogens in 4 patients, expressed as absolute numbers and percentages of the total…….. 30

Table 3.14: 12 microorganisms identified on culture as one of four pathogens in 3 patients, expressed as absolute numbers and percentages of the total…….. 31

Table 3.15: Comparison of frequency of isolation of different microbiological categories of microorganisms between clusters of patients with different numbers of microorganisms identified……31

Table 3.16: Distribution of 57 identified microorganisms in 33 male and 19 female patients…… 34

Table 3.17: Extraction of recorded microbial sensitivity & resistance profiles per antibiotics…… 36

Table 3.18: Reported antibiotic sensitivity profiles of microorganisms……37

Table 3.19: Reported antibiotic sensitivity profiles of microorganisms (continuation of Table 3.18 above)……..38

Table 3.20: Reported antibiotic sensitivity profiles of microorganisms (continuation of Table 3.19 above)……..39

Table 3.21: Reported antibiotic resistance profiles of microorganisms……40

Table 3.22: An overview of reported number of cases of microbial antibiotic resistance……47

Table 3.23: Amoxicillin – clavulanic acid sensitivity trend for microorganisms…….. 57

Table 3.24: Amoxicillin – clavulanic acid resistance trend for microorganisms……..58

Table 3.25: Histology distribution: Categorisation of patients' specimen results……61

Table 3.26: Tissue necrosis distribution: Categorisation of patients' specimen results.....62

Table 3.27: TB investigations in DNIs.....68

Table 3.28: TB investigations in DNIs: Histology & staining……..68

Table 3.29: Fungal investigations in DNIs…….72

NOMENCLATURE

ACIBA **:** *Acinetobacter baumannii*

BACSP **:** *Bacillus* species

CANAL **:** *Candida albicans*

CHBAH **:** Chris Hani Baragwanath Academic Hospital

CITFR **:** *Citrobacter freundii*

CMJAH **:** Charlotte Maxeke Johannesburg Academic Hospital

CNS **:** *Coagulase negative staphylococcocus*

CORSP **:** *Corynebacterium* species

CT **:** computerized tomography

DNI **:** Deep neck infection

ENCFE **:** *Enterococcus faecium*

ENT **:** ear, nose and throat

ENTCL **:** *Enterobacter cloacae*

ENTFA **:** *Enterococcus faecalis*

ESBL KLESP: Extended spectrum beta lactamase producing *Klebsiella*

ESCCO **:** *Escherichia coli*

GPB 1+ **:** Gram positive bacilli - Scanty (1+)

GPB 2+ **:** Gram positive bacilli - Moderate (2+)

GPC 1+ **:** Gram positive cocci – Scanty (1+)

GPC 2+ **:** Gram positive cocci - Moderate (2+)

GPC 3+ **:** Gram positive cocci - Numerous (3+)

GNB 1+ **:** Gram negative bacilli - Scanty (1+)

GNB 2+ **:** Gram negative bacilli - Moderate (2+)

GNB 3+ **:** Gram negative bacilli - Numerous (3+)

GNB (mix) **:** Gram negative bacilli (mixed enteric)

HAEPA **:** *Haemophilus parainfluenzae*

KLESP **:** *Klebsiella*

MC&S **:** microbial, culture and sensitivity results

MMED **:** Masters in medicine

MOGMO **:** *Morganella morganii*

MRSA **:** Methicillin resistant *Staphylococcus aureus*

MTB **:** *Mycobacterium tuberculosis*

NHLS **:** National Health Laboratory Services

ORL-HNS **:** Otorhinolaryngology- Head and Neck Surgery

PCR **:** Polymerase chain reaction

S. aureus **:** *Staphylococcus aureus*

SALSP **:** *Salmonella* species

SERMA **:** *Serratia marcescens*

SERSP **:** *Serratia* species

SMG **:** *Streptococcus milleri* group

STAAU **:** *Staphylococcus aureus*

STAEP **:** *Staphylococcus epidermidis*

STAHA **:** *Staphylococcus haemolyticus*

STEMA **:** *Stenotrophomonas maltophilia*

STRAN **:** *Streptococcus anginosus*

STRGF **:** *Streptococcus Group F*

STRMI **:** *Streptococcus milleri*

STRPY **:** *Streptococcus pyogenes*

STRSP **:** *Streptococcus* species

STRVI **:** *Streptococcus viridans*

TB **:** Tuberculosis

1 INTRODUCTION

Deep neck spaces are regions of loose connective tissue filling areas between the three layers of deep cervical fascia, namely superficial, middle and deep layers. The superficial layer is the investing layer. The pretracheal layer is the intermediate layer and the prevertebral layer is the most deep layer.[1] Deep neck spaces are classified into three anatomic groups, relative to the hyoid bone:

- Those located above the level of the hyoid (peritonsillar, submandibular, parapharyngeal, masticator/temporal, buccal, and parotid spaces)
- Those that involve the entire length of the neck (retropharyngeal, prevertebral, and carotid spaces)
- The anterior visceral, or pretracheal space located below the hyoid.[2]

Deep neck infection (DNI) is defined as infection in the potential spaces and actual fascial planes of the neck.[3] Once the natural resistance of fascial planes are overcome, spread of infection occurs along communicating fascial boundries.[2] Patterns of spread occur as abscess formation, cellulitis and necrotizing fasciitis.[4] Different classification systems for DNIs exist in the literature: One example is that of specifically categorizing deep neck abscesses into six groups according to abscess location to clarify the causal relationship and complication risk.[5]

1.1 Literature review

Since the discovery of penicillin, the widespread use of antibiotics has dramatically reduced the incidence of DNIs.[6] More recent trends include the increasing prevalence of resistant bacterial strains, a decline in DNIs caused by pharyngitis or

tonsillitis, and a relative increase in DNIs of odontogenic origin.[7] Low socioeconomic status and poor oral hygiene have been associated with higher rates of odontogenic infections, including Ludwig's angina.[2] Moreover, there is an increase in global obesity as part of the metabolic syndrome. Patients with diabetes mellitus and HIV infection are at risk for atypical and more complicated DNIs.[2] Hence, the prevalence pattern of bacteria is possibly changing, and if so, the initial empiric antibiotic therapy for DNIs must appropriately be changed, to remain effective.

Previous MMED studies conducted at CHBAH and allied hospitals have used the population of greater Johannesburg and Soweto for their retrospective microbiology review. Of note, is a prospective MMED comparative study of potential risk factors between Ludwig's angina and localised odontogenic abscesses. The study was done on patients presenting to the maxillo-facial surgery clinics, and casualties at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Chris Hani Baragwanath Academic Hospital (CHBAH). The purpose of the study was, amongst others, also to compare the micro-organisms involved in the two infections. Findings were that highly virulent bacteria such as *Staph aureus* and *Porphyromonas/ Prevotella* are responsible for the infection that develops in Ludwig's angina. Anaerobic bacteria isolated from Ludwig's angina were resistant to erythromycin and aerobic bacteria, to carbinicillin.[8]

The most frequent DNI is peritonsillar abscess, followed by submandibular, parotid, parapharyngeal, retropharyngeal, masseteric and pterygomaxillary abscesses and finally Ludwig's angina.[9]This is in contrast to a report of a decline in DNIs caused by tonsillitis or pharyngitis.[2] Nevertheless, in most cases, the source of the infection is a

periapical infection of the mandibular second or third tooth i.e. of odontogenic origin, or tonsillitis.[1] A case report on a patient with a left submandibular abscess with underlying tongue squamous cell carcinoma and neck metastasis has been published.[10] Also reported in the literature, is that an estimate of 22% of DNIs in adults, are idiopathic.[9]

The microbiology of DNIs are similar, reflecting normal endogenous upper aerodigestive tract flora. Thus no correlation usually exists between the anatomic site and microbiology of infection.[11] Of note however, is infection in the prevertebral space. Spread of infection to this space can be primarily haematogenous and thus their pathogenesis and microbiology is different, usually a predominance of *Staphylococcus aureus* and anaerobes playing a minor role. Special mention of TB infection in Pott's disease can result in complications such as local vertebrae or disc destruction with spinal instability, or a psoas abscess distally.[12] Coaggregation of microbes promotes plaque biofilm formation on the tooth surface. The microbial shift that occurs with plague progression and various associated odontogenic infections have been described.[1]

Most DNIs are polymicrobial. Only 5% are purely aerobic and 25% with isolated anaerobes.[4] From a resource perspective, the utility of cultures have been questioned, with some reports indicating that culture and sensitivity data do not lead to a change in antibiotic selection or treatment.[4] Recommendations for cultures include extensive or rapidly spreading infections, necrotizing and gas-forming infections, nosocomial or recurrent infections, and in the immuno-compromised host.[4]

The use of cultures is also supported due to the ever increasing possibility of drug-resistant organisms.[4]

The number of negative or contaminated cultures are not negligible, in the literature. One study reported 18.8% of cultures, as negative and 1.1%, as contaminated.[9] Negative cultures may be due to high dose of IV antibiotics prior to surgical drainage of DNIs.[3] Errors during sample collection, as occur with anaerobes when the sample is taken using a syringe containing air, may also be a contributing factor.[9] Of note is that anaerobes are fastidious and thus difficult to culture and are often overlooked. Their exact role in disease is difficult to ascertain due to inconsistent methods used for isolation and identification.[4] Some studies concur with the above.[1] However, in an analysis of 233 patients with DNIs, gram positive anaerobic cocci were most often isolated.[14] Blood cultures have a low positive rate of 15.5% and are thus assumed to have little impact on the management of deep neck abscesses, in particular, as compared to pus cultures.[3] Molecular techniques, e.g. sequence-based analysis, such as 16S rRNA, have been advocated for organisms that are poorly cultivable or for identification when traditional phenotypic methods have failed. [15]

Earlier studies emphasised *Streptococcus pyogenes, Staphylococcus aureus* and anaerobes as predominant organisms in DNIs.[16] Although anaerobic cultures were not done in all cases , one study reported that gram negative organisms have replaced haemolytic *Streptococcus* as the dominant pathogen in deep neck abscesses.[16]

In a study of 365 cases of DNIs, *Streptococcus viridans*, Coagulase-negative *staphylococcus, Staphylococcus aureus* and *Klebsiella pneumoniae* were found to be the most prevalent aerobic organisms. *Bacteroides*, *Peptostreptococcus, Fusobacterium* and *Prevotella* were the most prevalent anaerobes, in descending order respectively.[1] Another study reported similar results in an analysis of 634 patients.[17] Factors predictive of severe DNIs are streptococcus infections, presence of tonsils, education level and geographic location.[18]

Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) isolation is increasingly common, especially in IV drug abusers and immune-compromised patients and may play an increasing role in DNIs in the near future.[1] This organism is recognised as a nasal coloniser.[12] One study reports on a patient, with MRSA necrotising fasciitis that originated at the base of the nose, who eventually had a reconstruction with a radial forearm free flap.[4] In certain geographical areas MRSA is an emerging pathogen in necrotising fasciitis and descending mediastinitis.[2] Of interest, is a case report of an 88 year old man with a mycotic pseudoaneurysm of the common carotid artery mimicking a parapharyngeal abscess. The causative organism was community acquired MRSA.[19]

Klebsiella pneumonia is the most common causative pathogen in diabetic patients with DNIs.[3] Diabetes mellitus and multiple deep neck space involvement are the strongest independent predictors of complications.[1] In another study, the *Streptococcus milleri group* (SMG) was identified in 33% of DNIs. Of note is that the SMG infection spreads rapidly and is also suspected to be involved in systematic purulent diseases e.g. empyema, hepatic and cerebral abscesses.[5] Lemierre's

syndrome (septic internal jugular vein thrombophlebitis) complicates 1% or less of DNIs, most commonly in young adults. Approximately 80% of cases are associated with *Fusobacterium necrophorum* bacteremia.[21] An analysis of 118 case reports found that the commonest metastatic focus is the lung followed by joints. The overall mortality was 6%.[21] In patients with deep neck abscesses, factors related to mortality were: Multiple deep neck space involvement ($p < 0.01$), bilateral involvement ($p < 0.05$) and reoperation ($p < 0.001$); 40 % of these patients had comorbidities, with diabetes mellitus being the commonest, found in 34% of patients.[20]

With regards to the influence of the patient's age on DNIs, odontogenic and salivary origin are the most common sources of infection for the elderly (>65 years of age). Compared to the adult group (>18 years of age), elderly patients with DNIs had more multiple space involvement, complications, surgical interventions and longer hospital stays. However, outcome in the two groups were the same. Therefore aggressive management for DNI should not be withheld simply because of old age.[22]

The role of atypical organisms in DNIs also needs mention. One study reported TB as a cause of DNI in 6.9% of patients.[23] A case report of DNI caused by *Salmonella enterica* in a 46 year old man newly diagnosed with type II diabetes mellitus, has been published. The mechanism of spread is either haematogenous or lymphatic from a primary gastrointestinal infection.[24]

Empiric antibiotic therapy with a combination of penicillin plus a beta-lactamase inhibitor e.g. amoxicillin/clavulanate, is recommended among other options, providing sufficient coverage for both anaerobic and aerobic bacteria.[1] This is the current

practice at CHBAH. Clindamycin may no longer be considered a first-line antibiotic in DNIs, as current resistance rates among *Bacteroides fragilis* strains reach 20-50% or more, worldwide.[1] Surgical exploration and drainage of DNIs may be mandatory in selected cases at presentation or in cases that fail to respond to parenteral antibiotics within the first 24 to 48 hours. Cultures should be done intra-operatively to establish the pathogen(s) involved and to obtain an antibiogram to tailor the antibiotic treatment.[14]

Patients with DNI are referred to the department of Otorhinolaryngology at CHBAH, usually when surgical intervention is anticipated. Prompt medical treatment, with or without surgery, is usually initiated. Each patient is given initial empiric antibiotic therapy pending DNI microbiological, culture and sensitivity (MC&S) result availability from the National Health Laboratory Service (NHLS), when requested.

Empiric therapy is based on suspected rather than known pathogens whose antimicrobial susceptibility patterns are predictable.[25] Observed differences in the local prevalence of bacterial pathogens and the continued emergence of antibiotic-resistant strains of bacteria influence the choice of antibiotic used to initiate treatment.[26,27] This noted trend of the increasing prevalence of more resistant bacterial strains is the reason for this study: an audit of the prevalent bacteria associated with DNI is required to ascertain if the current empiric antibiotic therapy in use at CHBAH is effective. In essence, continued periodic surveillance testing on local susceptibility patterns within specific health care centres is warranted.[15]

In recent years, the incidence of DNIs has increased, but mortality has declined.[9]This has been attributed to a comprehensive, patient centred multidisciplinary approach, including improvements in diagnostic and therapeutic interventions, namely: airway control, radiological imaging, effective antibiotic usage, early surgical intervention, microbiology and critical care support, respectively.[1, 3,]

1.2 Hypothesis:

The empiric antibiotic therapy currently in use is effective against microbes commonly involved in DNIs in adults at CHBAH.

1.2.1 Study objectives

The aim of the study is:

- To determine if micro-organisms are identified in DNIs.
- To review the types of micro-organisms commonly isolated from DNIs.
- To determine the antibiotic sensitivity and resistance of these micro-organisms.
- To make recommendations on the choice of antibiotics to be used to initiate treatment prior to obtaining MC&S results from the NHLS.

2 METHODOLOGY

This was a quantitative research study.

2.1 Study Design

This study was a retrospective review of the microbiology of DNIs.

2.2 Study Population

This study included all patients with DNIs from whom specimen microbiology samples were taken, comprising:

- **Inclusion criteria**
    - All adult patients 18 years or older
    - Diagnosed with DNI
    - Required surgical intervention
    - For whom DNI MC&S were obtained.

- **Exclusion criteria**

  All patients with DNI:
    - Extended into the mediastinum on CT – these patients were transferred for surgery to another hospital which, unlike CHBAH, had cardiothoracic surgical staff.

- Who had only active medical management of DNI with/without aspirates submitted for microbial analysis, ie not intra-operatively
- Who had surgery performed by other surgical departments.

2.3 Study Location

The otorhinolaryngology clinical units of CHBAH in Gauteng. This is a tertiary referral hospital affiliated to the University of the Witwatersrand.

2.4 Study Period

The study extended over a period of 7 years, from 01/07/08 to 30/06/15.

2.5 Data Collection

Patients were identified from three sources:

- ENT operating theatre register
- ENT ward registers (admissions, discharge and mortality)
- Radiology CT scan registers

The operating theatre register was used as the primary reference to identify patients. The ENT ward registers and the radiology CT scan registers were used as secondary references to identify patients, whose theatre records were inadequate (misspelt names/ incorrect hospital numbers, or whose records were illegible at places).The secondary references were used as a screen to source for patients whose

information had not been identified specifically in the ENT theatre register, e.g. patients whose first surgical procedures were recorded but not their relook procedures; and vice versa as well as for those patients for whom the first surgical procedures and relook procedures were not recorded in the theatre register.

Eligible patients correctly identified from the theatre register were recorded as an initial sample population. This information, i.e., patients' hospital numbers/full names, was then used to search the NHLS database for the results of specimens sent for MC&S. Requested histology reports were also obtained. The NHLS database was accessed online, either via a personal or hospital owned computer; with a personal username and password. Thus the names and records obtained from the NHLS database was password protected, remained confidential for ethical purposes. The relevant information was extracted and all findings were recorded into one of the following categories:

- Positive culture – micro-organism/s were identified, i.e growth
- Negative culture – the report stated "no bacterial growth"
- No results – no specimen MC&S results were found in the database
- Rejected specimens – these specimens were unsuitable for processing
- Incomplete or incorrect information – not sufficient information was available from the registers to search the NHLS database or the NHLS database information *per se* was not found to be useful.
- Histology results – a histological analysis, not a microbiological analysis, was done on the specimen.

Patients identified exclusively from the secondary references were cross-checked against their recorded microbiological laboratory reports, and were included in the study population. If “rejected specimens”, “no results” or “incomplete or incorrect information” was found, then these were excluded from the final study population.

The following were recorded, for each patient, on the data collection sheets (see appendix IA, IB):

- Hospital registration number (This was kept confidential- did not appear in the data collection sheets submitted in this final research report.)
- Name & Surname. (This was kept confidential)
- Date of surgery for DNI
- Gender
- Age
- Type of isolated microorganism
- Antibiotic sensitivity & resistance
- Histological analysis done, or not
- Tissue necrosis reported on histological analysis, or not

All data was captured on Microsoft Excel Spreadsheet.

2.6 Ethical Considerations

All patient information was kept strictly confidential. Ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand. The Ethics Certificate was presented as appendix II. No patient consent was required, as this was a retrospective review.

2.7 Problems and Potential Limitations

This study was conducted at CHBAH, which is the largest hospital in Africa: All information was not meticulously captured and some data was collected from patient records that were not well managed, for example, files went missing at times. Thus accessing all information was at times laborious, as this was a retrospective data collection process.

A sample size of 50-55 patients with DNIs, who fell into the inclusion category of data collection; was given. No significant findings were possible due to this small size. To accommodate inadequate patient records and patient numbers, the initial 3 year study period from 01/07/'12 – 30/06/'15 was prolonged retrospectively to 01/07/'08. This 7 year study period was to ensure that the minimum target sample population of 50-55 patients, was obtained. However, a sample size calculation was not necessary for this retrospective audit, over a fixed time period. Not all patients with DNIs were accounted for: Those treated with active medical management only; those with more advanced disease, e.g. mediastinitis; or patients managed by other departments, e.g. DNI of odontogenic origin treated by Maxillo-facial surgery staff; thus a selection bias was favoured.

2.8 Data Analysis

Standard statistical methods were used for analysis. To determine the statistical significance of findings, probability values were calculated. A probability value of

$p < \text{or} = 0.05$ was regarded as significant. Statistical values such as mean, median, range, minimum, maximum, standard deviation and standard error etc. were calculated for age, gender, antibiotic sensitivity/resistance patterns and histograms were drawn. The bacterial profile in male patients were compared to female patients for various variables as seen in the tables, and its statistical significance was determined. Chi Square test and Student's t-test were used to determine statistical significance.

Use was made of the following software:

- Microsoft Word 2013 Word Processor for all typing requirements.
- Microsoft Excel 2013 Spreadsheet for data storage, manipulations and analysis. It was also used for statistical calculations, as mentioned above.
- Web Chi Square calculator for comparison of sample population.[28]

3 RESULTS AND ASSOCIATED DISCUSSION

3.1 Analysis of the Study Population and Study Period

Analysis of the study population:

For the three year study period 01/07/'12 – 30/06/'15, a total of 63 adult patients with DNIs were obtained from the registers. The 63 patient hospital clinical records were then requested for, and DNI inclusion criteria were verified as per clinical findings, radiological investigations and or theatre notes captured:

There were 6 patient records not accessible. A total of 57 patient hospital clinical records were accessed. From these records, 33 patients met inclusion criteria and 24 patients were thus excluded.

From the 24 patients excluded:

- 17 patients did not have DNIs
- 6 patients did not have MC&S specimens submitted to the NHLS or MC&S results were not available in the NHLS database
- 1 patient was not an adult with DNI

For the 6 patients whose records were not accessible:

After cross-checking patient register details against their NHLS reports, 2 patients met inclusion criteria. Thus 4 patients were excluded due to no hospital clinical records being available.

In total, 28 patients were excluded. In total, 35 patients were included. These patients contributed 47 specimen MC&S results from initial and relook surgical procedures.

The study period was prolonged retrospectively to 01/07/08; to ensure that 50 – 55 patients with DNIs were included in the study, as per protocol.

For the 4 year study period 01/07/'08 – 30/06/'12, a total of 32 adult patients with DNIs, at CHBAH, were obtained from the registers. The 32 patient hospital clinical records were then requested for, and DNI inclusion criteria were verified as per clinical findings, radiological investigations and or theatre notes captured: There were 4 patient records not accessible. A total of 28 patient hospital clinical records were accessed. From these records, 17 patients met inclusion criteria, thus 11 patients were excluded.

From the 11 patients excluded:

- 3 patients did not have DNIs
- 4 patients did not have MC&S specimens submitted to the NHLS or MC&S results were not available in the NHLS database
- 4 patients had surgeries performed for DNIs by other surgical departments

In total, 15 patients were excluded. The 17 patients included, contributed 23 specimen MC&S results from initial and relook surgical procedures.

For the seven year study period 01/07/'08 – 30/06/'15, a total of:

95 patients (63+32) : From registers

8 patients (4 + 4) : Records not accessed

87 patients (59+28) : Records accessed

43 patients [(24+4) + (11+4)] : Excluded

52 patients [(33+2) + (17)] : Included

Analysis of patient admissions over the study period (01/07/2008 – 30/06/2015)

Table 3.1 52 patient admissions over the 7 year study period (01/07/2008 – 30/06/2015)

Year No.	Duration	No. of Patients
1	01/07/2008-30/06/2009	6
2	01/07/2009-30/06/2010	4
3	01/07/2010-30/06/2011	6
4	01/07/2011-30/06/2012	1
5	01/07/2012-30/06/2013	8
6	01/07/2013-30/06/2014	9
7	01/07/2014-30/06/2015	18

During the initial 3 years of the study period (01/07/2008 – 30/06/2011), patient admissions were relatively constant, varying between 4 and 6 patients per year. In the fourth year of the study period (01/07/2011 – 30/06/2012), patient admissions noticeably declined, followed by a significant increase in patient admissions during the final 3 years of the study period (01/07/2012 – 30/06/2015) ($p<0.05$); with an absolute doubling in patient admissions during the final year (01/07/2014 – 30/06/2015) relative to the preceding year (01/07/2013 – 30/06/2014).

3.1.1 Analysis of age distribution of 52 patients

Table 3.2 Age statistics of all patients

Statistic	**Value**
Mean	40.9
Median	39
Mode	28
Std. Deviation	15.5
Kurtosis	-0.61
Skewness	0.5
Minimum	19
Maximum	77
Count	52

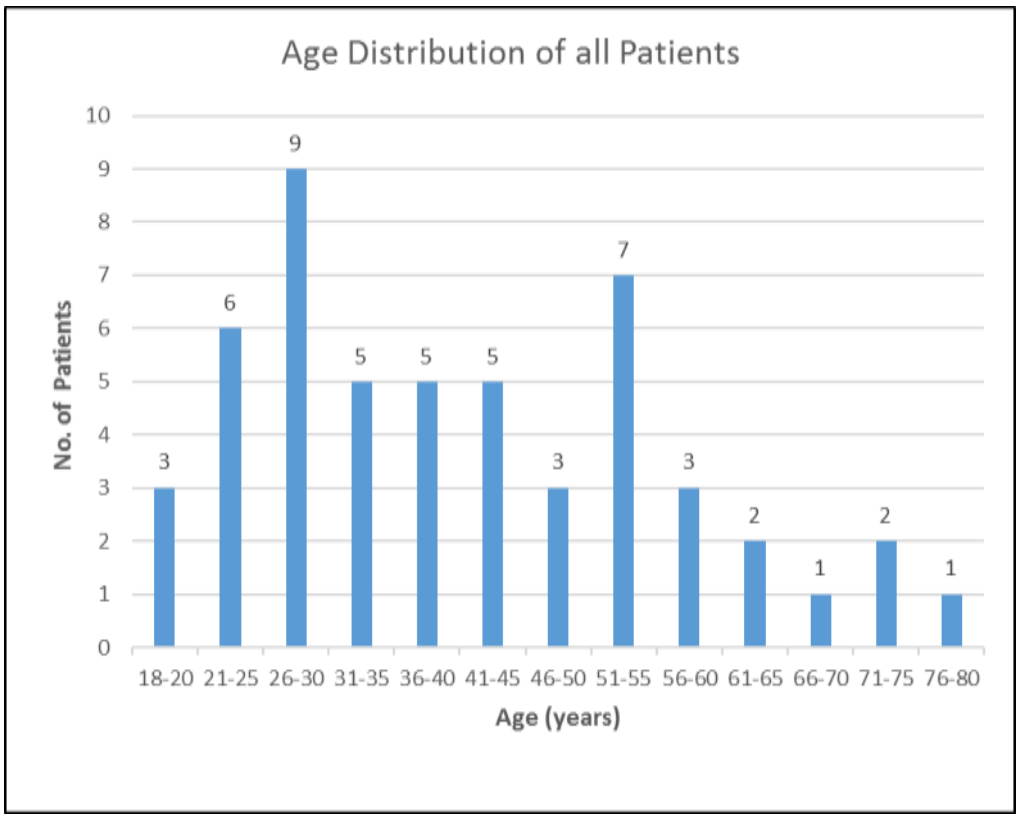

Figure 3.1 Age distribution of 52 patients

The age distribution of patients ranged from 19 to 77 years with a mean of 40.9 (+ - 15.5) yrs.

3.1.2 Analysis of gender distribution of 52 patients

Table 3.3 Gender distribution of all patients

Males	33	63%
Females	19	37%
Total	52	100%

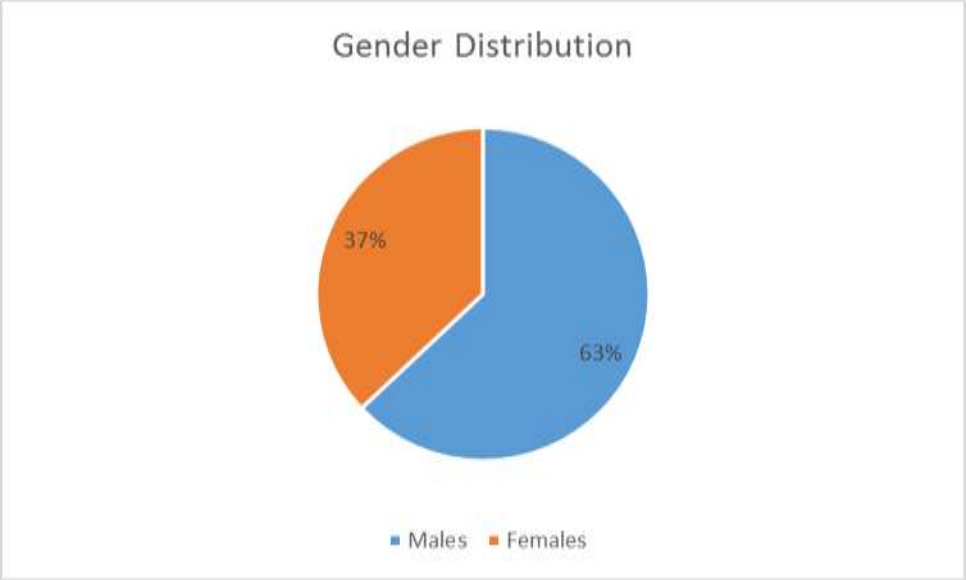

Figure 3.2 Gender distribution of 52 patients

The study population comprises 63% (n=33) males and 37% (n=19) females. The ratio of males: females is 1.74:1.

3.1.3 Analysis of 70 specimen microbiological cultures in patients with DNIs

The 52 patients contributed 70 (47 + 23) specimen MC&S results from initial and relook surgical procedures. In addition, some patients had more than one specimen MC&S result per surgical procedure for DNI. One specimen was categorized as a 'rejected specimen'- due to a lab error. This is depicted with " – " in Appendix IA. For 10 (6+4) patients, specimens were categorised as 'no results found'. No specimen was categorized as 'incomplete or incorrect information'.

Table 3.4 Categorization of microbiological culture results from 70 specimens of 52 patients with DNIs

Result category	**No. of specimens**	**%**
Positive cultures	48	69
Negative cultures	22	31
Total	**70**	**100**

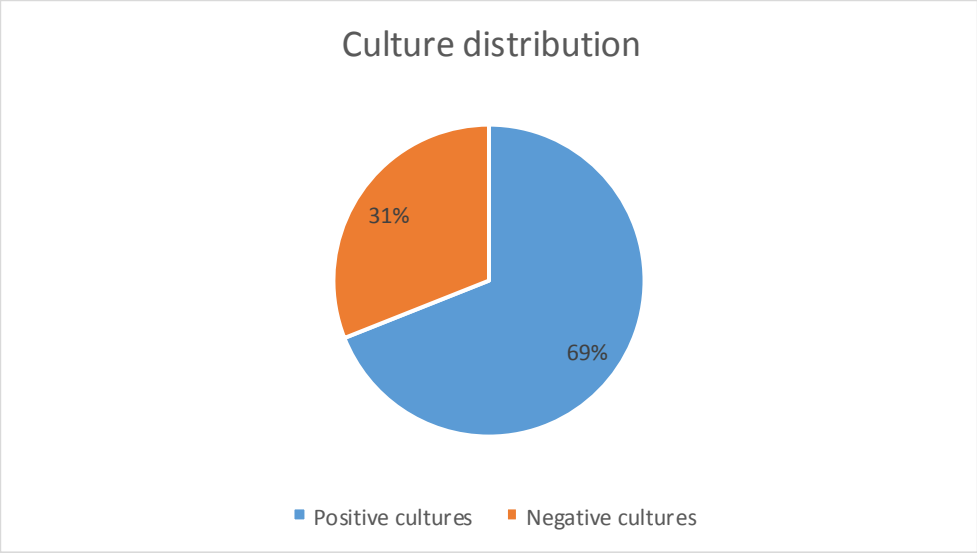

Figure 3.3 Culture distribution of specimens

The distribution of positive cultures is 69% (n=48) and that of negative cultures is 31% (n=22), from 70 specimens of 52 patients with DNIs. This percentage of negative cultures is not negligible; higher than that quoted in some studies and lower than that in others, 19% and 52% respectively.[1,9] Possible explanations include

errors in sample collection and processing, fastidious growth patterns and pre – operative empiric antibiotic usage.[1,3,4,9]

.

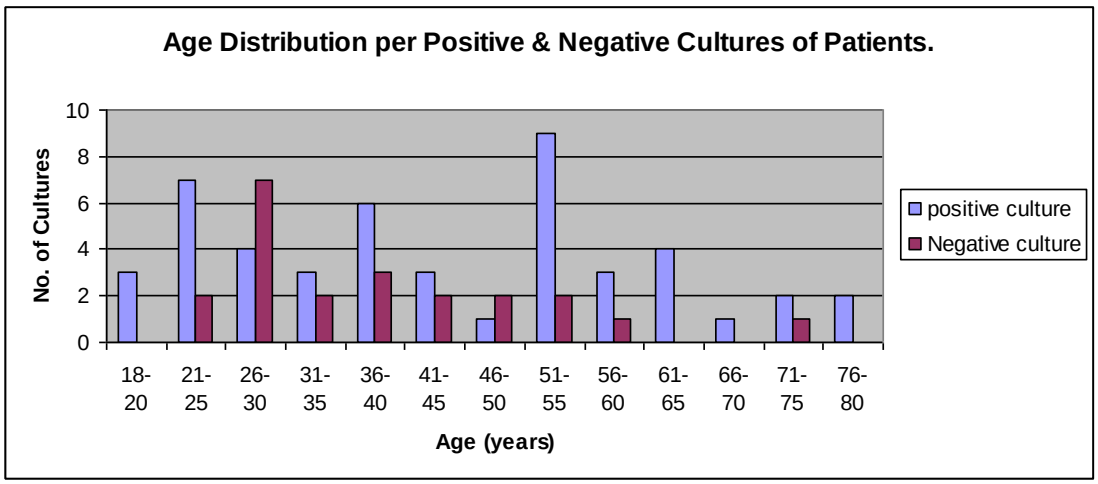

Figure 3.4 Age distribution per positive & negative cultures of patients

The age distribution per positive cultures of patients is that of 69% (n=33) between the ages of 21 and 55 with a peak incidence of 19% (n=9) in the age category 51-55, followed by a second peak incidence of 15% (n=7) in the age category 21-25. The age distribution per negative cultures of patients is that of 82% (n=18) between the ages of 26 and 55 with a peak incidence of 32% (n=7) in the age category 26-30. The Student's t-test for age distribution per positive and negative cultures of patients revealed no significant difference ($p>0.05$).

Table 3.5 Gender distribution per positive & negative cultures of patients

		Subtotals	**Totals**
FEMALE PATIENTS	**No. of patient specimens**		
POSITIVE CULTURES:			
- initial surgical procedure	12		
Additional specimens:			
- initial surgical procedure	4		
- relook surgical procedure	1	17	
NEGATIVE CULTURES:			
- initial surgical procedure	7		
Additional specimens:			
- initial surgical procedure	1		
- relook surgical procedure	0	8	25
MALE PATIENTS			
POSITIVE CULTURES:			
- initial surgical procedure	22		
Additional specimens:			
- initial surgical procedure	3		
- relook surgical procedure	6	31	
NEGATIVE CULTURES:			
- initial surgical procedure	11		
Additional specimens:			
- initial surgical procedure	3		
- relook surgical procedure	0	14	45
TOTAL			70

Table 3.6 Gender distribution per positive & negative cultures of patients: Categorisation of the results from 70 specimens

Result category	**No. of patient specimens**	**%**
Females: Positive cultures	17	24.3
Females: Negative cultures	8	11.4
Males: Positive cultures	31	44.3
Males: Negative cultures	14	20
Total	**70**	**100%**

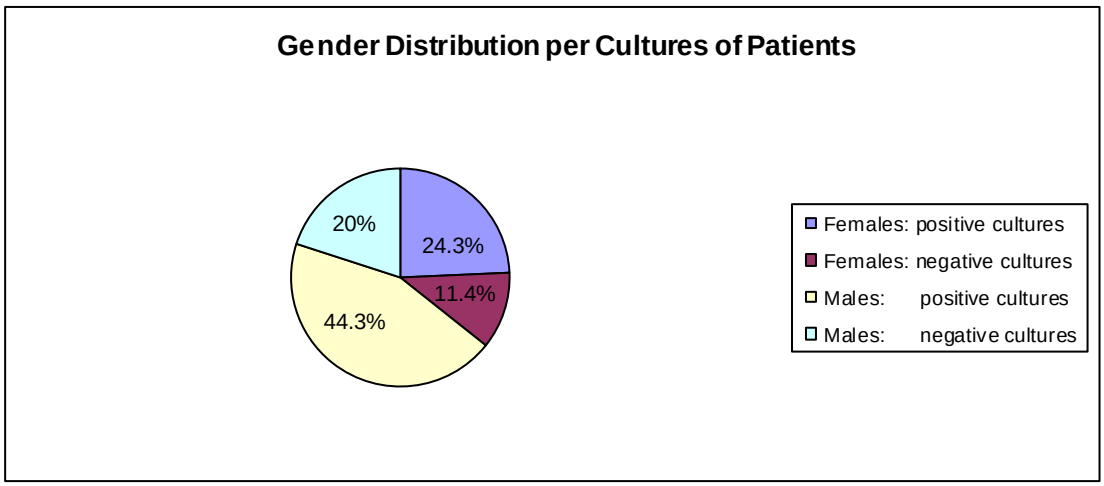

Figure 3.5 Gender distribution per positive & negative cultures of patients

The gender distribution per positive cultures of patients comprises 24.3% (n=17) females and 44.3% (n=31) males. The gender distribution per negative cultures of

patients comprises 11.4% (n=8) females and 20% (n=14) males. The Student's t-test for gender distribution per positive and negative cultures of patients revealed no significant difference ($p>0.05$).

3.2 Analysis of Microbiological Cultures per Absolute Patient Count

Table 3.7 Categorisation of microbiological culture results in 52 patients; per absolute patient count

Result category	**No. of patients**	**%**
Positive cultures	36	69
Negative cultures	16	31
Total	**52**	**100**

The distribution of positive cultures is 69% (n=36) and that of negative cultures is 31% (n=16), per absolute count of 52 patients with DNIs.

An aerobic gram positive coccus was isolated in 1 patient with a positive culture, however, the microorganism was non-viable (Appendix 1A) and will thus be preferably excluded: Total number of patients with positive cultures will be depicted as *35 in calculations to follow.

Table 3.8. Comparison of frequency of isolation of different microbiological categories of microorganisms in *35 patients with positive cultures; per absolute patient count

Microbiological category	**Frequency**	**%**
Aerobes only	31	89
Aerobes & Anaerobes	4	11
Anaerobes only	0	0
Total	***35**	**100%**

Table 3.9 Number of different microorganisms identified per patient distribution, expressed as absolute numbers and percentages of the total study population with positive cultures

No. of different microorganisms per patient	**No. of patients**	**% of patient total**
1	23	66
2	5	14
3	4	11
4	3	9
Total	***35**	**100%**

Table 3.10 All pathogens: 57 microorganisms identified on culture in 35 patients, expressed as absolute numbers and percentages of the total

Microorganisms	**No.**	**% of Total**
Aerobes		
ACIBA	6	10.5
STRPY	4	7
STRAN	4	7
CNS	3	5.3
STRGF	3	5.3
ENTFA/ENCFE	4	7
STRSP	2	3.5
STRMI	3	5.3
STAHA	2	3.5
MOGMO	1	1.8
KLESP	3	5.3
STRVI	2	3.5
ENTCL	2	3.5
CANAL	1	1.8
CITFR	1	1.8
CORSP	1	1.8
ESCCO	1	1.8
HAEPA	1	1.8
SALSP	2	3.5
SERSP/SERMA	2	3.5
AFB	1	1.8
STAEP	1	1.8
STAAU	2	3.5
STEMA	1	1.8
Anaerobes		
Prevotella	3	5.3
CORSP	1	1.8
TOTAL	**57**	**100**
Summary:		
Aerobes	**53**	**93**
Anaerobes	**4**	**7**

There were 26 different microorganisms isolated and identified on culture. Of these, 92% (n=24) of microorganisms were aerobic and 8% (n=2) anaerobic. The most frequently isolated aerobic microorganisms, in descending order, were *Acinetobacter baumannii* (ACIBA) ; *Streptococcus pyogenes* (STRPY), *Streptococcus anginosus* (STRAN), *Enterococcus faecalis / Enterococcus faecium* (ENTFA/ENCFE); *Coagulase negative staphylococcocus* (CNS), *Streptococcus Group F* (STRGF), *Streptococcus milleri* (STRMI), *Klebsiella* (KLESP); respectively. Prevotella was the most frequently isolated anaerobic microorganism, in keeping with the literature.[1,16,17]

Thus, when considered in isolation, gram negative bacilli (GNB) (ACIBA) were the dominant micro – organisms in DNIs, however when clustered, GNB : Streptococcus species approximated 1:1, which is not in keeping with the literature.[16] Aerobic : anaerobic isolation & identification of the above more frequently occurring micro – organisms were in the order of 10:1.

A total of 33% (1 in 3) of patients with KLESP DNIs had diabetes mellitus type II, and presented in a state of diabetic ketoacidosis. Although infrequently encountered, atypical micro – organisms in this patient population included fungi, TB and salmonella, in keeping with the literature.[23,24]

Table 3.11 23 microorganisms identified on culture as single pathogens in 23 patients, expressed as absolute numbers and percentages of the total

Microorganisms	**No.**	**% of Total**
Aerobes		
STRPY	3	13
STRAN	3	13
STRSP	2	8.7
MOGMO	1	4.4
STRGF	2	8.7
CANAL	1	4.4
CNS	1	4.4
ENTFA/ENCFE	1	4.4
STRVI	1	4.4
ESCCO	1	4.4
STRMI	1	4.4
SALSP	2	8.7
STAAU	2	8.7
STEMA	1	4.4
AFB	1	4.4
Anaerobes	**0**	**0**
TOTAL	**23**	**100**
Summary:		
Aerobes	23	100
Anaerobes	0	0

Table 3.12 10 microorganisms identified on culture as co-existing pathogens in 5 patients, expressed as absolute numbers and percentages of the total

Microorganisms	**No.**	**% of Total**
Aerobes:		
ACIBA	2	20
CITFR	1	10
CNS	1	10
ENTFA/ENCFE	1	10
ENTCL	1	10
SERSP/SERMA	1	10
STRMI	1	10
KLESP	**1**	**10**
Anaerobes:		
Prevotella	**1**	**10**
TOTAL	**10**	**100**
Summary:		
Aerobes	9	90
Anaerobes	1	10

Table 3.13 12 microorganisms identified on culture as one of three pathogens in 4 patients, expressed as absolute numbers and percentages of the total

Microorganisms	**No.**	**% of Total**
Aerobes:		
ACIBA	3	25
ENTFA/ENCFE	1	8.3
KLESP	2	16.7
HAEPA	1	8.3
STAHA	2	16.7
STRPY	1	8.3
STRVI	1	8.3
Anaerobes:		
Prevotella	**1**	**8.3**
TOTAL	**12**	**100**
Summary:		
Aerobes	11	91.7
Anaerobes	1	8.3

Table 3.14 12 microorganisms identified on culture as one of four pathogens in 3 patients, expressed as absolute numbers and percentages of the total

Microorganisms	No.	% of Total
Aerobes:		
ACIBA	1	8.3
CNS	1	8.3
CORSP	1	8.3
ENTFA/ENCFE	1	8.3
ENTCL	1	8.3
SERSP/SERMA	1	8.3
STAEP	1	8.3
STRAN	1	8.3
STRMI	1	8.3
STRGF	1	8.3
Anaerobes:		
Prevotella	1	8.3
CORSP	1	8.3
TOTAL	**12**	**100**
Summary:		
Aerobes	10	83
Anaerobes	2	16.6

Table 3.15 Comparison of frequency of isolation of different microbiological categories of microorganisms between clusters of patients with different numbers of microorganisms identified

No. of different microorganisms per patient	Aerobes	Anaerobes	Total
1	23	0	**23**
2	9	1	**10**
3	10	2	**12**
4	10	2	**12**
TOTAL	**52**	**5**	**57**

Using the Chi Squared Distribution, a significant difference ($p < 0.05$) was found in the relative distribution of aerobes and anaerobes amongst the above 4 clusters of patients, including a comparison of monomicrobial relative to polymicrobial growth identification. This is most probably due to only aerobic microorganisms (n=23) identified in monomicrobial growth patterns without any anaerobic growth (n=0), whilst in polymicrobial growth patterns, a higher proportion of aerobic (n=29) relative to anaerobic (n=5) microorganisms were identified.

Thus when all pathogens were considered, polymicrobial growth was more common than monomicrobial growth patterns; 60% (n=34) and 40% (n=23), respectively (Table 15), in keeping with the literature.[4] However, 66% (n=23) of patients with positive cultures had monomicrobial growth identification with polymicrobial growth in only 34% (n=12) (Table 9). In a study of 365 patients, 49% (n=177) of cultures were positive, with only 16% polymicrobial.[1] Of note, in this study of 35 patients with positive cultures, 89% (n=31) had only aerobic growth and in only 11% (n=4), polymicrobial growth of aerobes and anaerobes were identified (Table 8); which is not in keeping with some literature.[4] However, in another study of 634 patients, the bacteriology of DNIs was polymicrobial; 81% (n=514) of cultures were positive, only aerobes isolated in 39%, polymicrobial growth of aerobes and anaerobes in 32% and only anaerobes in 10%.[17]

In this study, when all pathogens were considered, 93% (n= 53) were aerobic and 7% (n=4) anaerobic (table 10). Whether this represents a true reflection of anaerobes involved in DNIs; or an underestimation secondary to intraoperative specimen collection, laboratory processing or culture methods; requires further

clarification.[1,4,9,14,16] The incidence of anaerobic bacteria is higher in adults, DNIs of dental origin and in non diabetic patients.[17]

Boscolo – Rizzo discussed that there has been an increase in anaerobic bacteremias with multiple - drug - resistant organisms; as there is an increasing population with multiple comorbidities and compromised immune systems; that anaerobes express virulent factors that may promote localised infection dissemination and by producing beta – lactamase, are able to protect themselves and other penicillin – susceptible organisms from the activity of penicillins. He emphasised the importance and described the correct method of anaerobic specimen collection, transport and processing.[1]

Table 3.16 Distribution of 57 identified microorganisms in 33 male and 19 female patients

Microorganisms	Males	Females
Aerobes:		
ACIBA	3	3
STRPY	2	2
STRAN	3	1
CNS	3	0
STRGF	3	0
ENTFA/ENCFE	2	2
STRSP	2	0
STRMI	1	2
STAHA	0	2
MOGMO	1	0
KLESP	1	2
STRVI	1	1
ENTCL	2	0
CANAL	0	1
CITFR	1	0
CORSP	0	1
ESCCO	1	0
HAEPA	0	1
SALSP	1	1
SERSP/SERMA	2	0
AFB	1	0
STAEP	0	1
STAAU	2	0
STEMA	1	0
Anaerobes:		
Prevotella	1	2
CORSP	0	1
TOTAL	**34**	**23**
Summary:		
Aerobes	33	20
Anaerobes	1	3

No significant difference ($p>0.05$) was found on comparative distribution of aerobes and anaerobes amongst male and female patients.

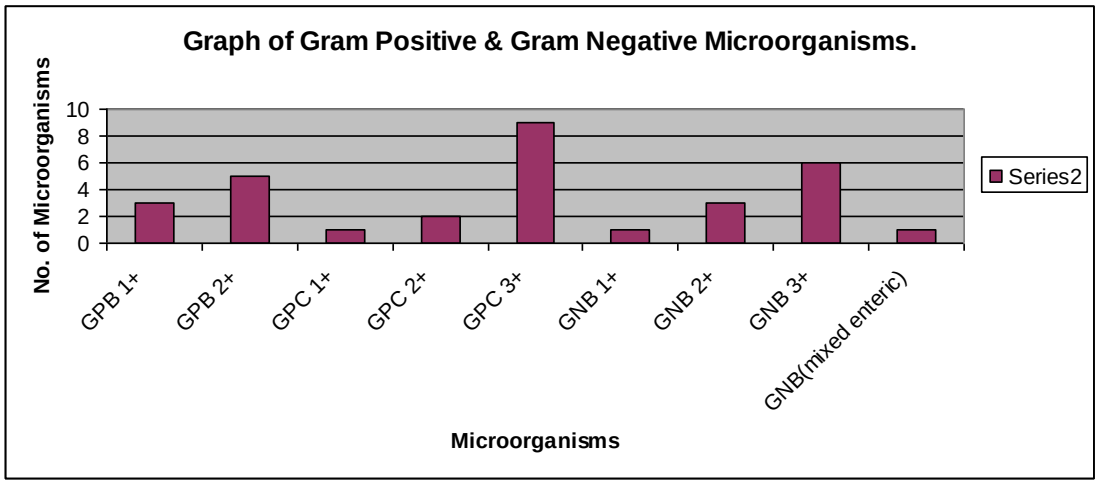

Figure 3.6 Comparative distribution of other gram positive and gram negative microorganisms isolated on microscopy only

Apart from the microorganisms isolated on microscopy and identified on culture, other gram positive cocci, gram negative bacilli and gram positive bacilli were isolated on microscopy, however not identified on culture (Figure 3.6 & Appendix 1B).

This is possibly attributed to the fastidiousness inherent to the microorganisms, particularly anaerobes.[4] Delays in timeous specimen analysis due to hospital resources constraints may have been contributory: Gram positive cocci 3+ were isolated on microscopy from 2 specimens of a patient however in both instances the microorganisms lost viability (Appendix 1B). Other probabilities include inadequate methods of specimen collection and transport or unfavourable culture mediums. Pre-operative intravenous antibiotic therapy duration could have also been instrumental. Notably, antibiotic sensitivity and resistance patterns are not reported for microorganisms isolated on microscopy and not identified on culture. This most likely has therapeutic implications on the correct usage of empiric and directed antibiotics or rather lack thereof in the medical management of patients with DNIs. The

significance of this in the context of not only diagnostic but also definitive therapeutic surgical management of patients with DNIs and its impact on mortality versus overall survival regardless, warrants further research. One could then also question the overall cost versus benefit of intra operative specimen collection for MC&S testing in patients with DNIs.

3.3 Antibiotic Sensitivity and Resistance Profiles of Microorganisms

Antibiotic sensitivity with or without resistance profiles of microorganisms were reported for 34 patients with positive cultures. No reports were documented for 2 patients who cultured *Candida albicans* (CANAL) and Gram positive cocci (non - viable), respectively (Appendix 1 B).

Table 3.17 Extraction of recorded microbial sensitivity & resistance profiles per antibiotics

Antibiotics	Reactive Microorganisms	Sensitivity	Resistance	Total
Ceftazidime	ACIBA	3 [43%]	4 [57%]	7 [100%]
Cefotaxime/ Ceftriaxone	KLESP	2 [67%]	1 [33%]	3 [100%]
Penicillin/ Ampicillin	CNS	2 [67%]	1 [33%]	3 [100%]
Amoxicillin-Clavulanic acid	KLESP	2 [67%]	1 [33%]	3 [100%]
Ciprofloxacin	ACIBA	2 [33%]	4 [67%]	6 [100%]

Table 3.18 Reported antibiotic sensitivity profiles of microorganisms

ANTIBIOTICS - SENSITIVITY	STAHA	STAEP	ENTFA/ ENCFE	ACIBA	KLESP	STAAU	CNS	ENTCL	SERMA/ SERSP
Penicillin/ Ampicillin			1 25%				2 50%		
Ampicillin/ Amoxicillin									
Cloxacillin						2 67%			
Piperacillin/ Tazobactam								1 20%	1 20%
Amoxicillin-Clavulanic acid					2 40%				
Cefotaxime/ Ceftriaxone (3rd generation Cephalosporin)					2 40%				
Ceftazidime (3rd generation Cephalosporin)				3 33.3%					
Cefepime (4th generation Cephalosporin)								2 40%	2 40%
Erythromycin/ Azithromycin						1 33%			
Clindamycin									
Ciprofloxacin				2 22.2%				1 20%	1 20%
Ertapenem					1 20%			1 20%	1 20%
Colistin				4 44.4%					
Vancomycin	2 100%	1 100%	3 75%				2 50%		
Trimethoprim-Sulfamethoxazole									
INH & Rifampicin									
Total	**2**	**1**	**4**	**9**	**5**	**3**	**4**	**5**	**5**

Table 3.19 Reported antibiotic sensitivity profiles of microorganisms (continuation of Table 3.18 above)

ANTIBIOTICS - SENSITIVITY	MOGMO	CITFR	SALSP	STRPY	STRAN	STEMA	STRSP	ESCCO
Penicillin/ Ampicillin				5 83%	4 100%			
Ampicillin/ Amoxicillin								
Cloxacillin								
Piperacillin/ Tazobactam	1 33.3%							
Amoxicillin-Clavulanic acid								1 100%
Cefotaxime/ Ceftriaxone (3rd generation Cephalosporin)			2 67%				2 100%	
Ceftazidime (3rd generation Cephalosporin)								
Cefepime (4th generation Cephalosporin	1 33.3%							
Erythromycin/ Azithromycin								
Clindamycin				1 17%				
Ciprofloxacin			1 33%					
Ertapenem	1 33.3%	1 100%						
Colistin								
Vancomycin								
Trimethoprim-Sulfamethoxazole						1 100%		
INH & Rifampicin								
Total	**3**	**1**	**3**	**6**	**4**	**1**	**2**	**1**

Table 3.20 Reported antibiotic sensitivity profiles of microorganisms (continuation of Table 3.19 above)

ANTIBIOTICS - SENSITIVITY	STRVI	HAEPA	MTB	STRMI	STRGF	Total
Penicillin/ Ampicillin	1 50%			3 75%	3 60%	18
Ampicillin/ Amoxicillin		1 100%				**1**
Cloxacillin						**2**
Piperacillin/ Tazobactam						**3**
Amoxicillin-Clavulanic acid						**3**
Cefotaxime/ Ceftriaxone (3rd generation Cephalosporin)	1 50%			1 25%		**8**
Ceftazidime (3rd generation Cephalosporin)						**3**
Cefepime (4th generation Cephalosporin						**5**
Erythromycin/ Azithromycin						**1**
Clindamycin					1 20%	**2**
Ciprofloxacin						**5**
Ertapenem						**5**
Colistin						**4**
Vancomycin					1 20%	**9**
Trimethoprim-Sulfamethoxazole						**1**
INH & Rifampicin			1 (100%)			**1**
Total	**2**	**1**	**1**	**4**	**5**	**72**

Table 3.21 Reported antibiotic resistance profiles of microorganisms

ANTIBIOTIC - RESISTANCE	STAHA	STAEP	ENTFA/ ENCFE	ACIBA	KLE-SP	STAAU	CNS	ENT-CL	SERMA/ SERSP	MOG-MO	CITFR	Total
Penicillin/	1	1				1	1					4
Ampicillin												
Ampicillin/			3		3			2	2	2	1	**13**
Amoxicillin												
Cloxacillin	2	1					1					**4**
Piperacillin/				8								**8**
Tazobactam												
Amoxicillin-					1			2	1	2	1	**7**
Clavulanic acid												
Cefazolin					1					1		**2**
(1st generation Cephalosporin)												
Cefuroxime					1						1	**2**
(2nd generation Cephalosporin)												
Cefotaxime/					1							**1**
Ceftriaxone (3rd generation Cephalosporin)												
Ceftazidime				4	1						1	**6**
(3rd generation Cephalosporin)												
Cefepime (4th generation				6	1							**7**
Cephalosporin)												
Erythromycin/		1										**1**
Azithromycin												
Clindamycin		1										**1**

Trimethoprim-		1		4	1					1		**7**
Sulfamethoxazole												
Ciprofloxacin				4								**4**
Imipenem				7								**7**
Meropenem				8								**8**
Tobramycin				2								**2**
Amikacin				1								**1**
Gentamicin				**4**						**1**		**5**
Total	**3**	**5**	**3**	**48**	**10**	**1**	**2**	**4**	**3**	**7**	**4**	**90**

For commonly isolated microbes with recorded incidences of sensitivity and resistance to a specific antibiotic, reported suggestions of directed therapeutic usage of antibiotics could be used to infer correct empiric antibiotic usage recommendations, retrospectively. Although empiric antibiotic usage guidelines cannot be based on this study solely, the following inferences were made:

Cefotaxime/Ceftriaxone (third generation cephalosporins) are suitable alternatives to amoxicillin – clavulanic acid in treating patients with DNIs caused by *Klebsiella* (KLESP), as their susceptibility and resistance profiles are equivalent, 67% and 33% respectively. In practical terms, this is also a consideration when amoxicillin – clavulanic acid is out of stock, as occasionally does occur in resources stretched tertiary hospitals such as CHBAH. Penicillin/Ampicillin may be used in the treatment of patients with DNIs caused by *Coagulase negative staphylococcocus* (CNS), with a reported sensitivity of 67%. One reported case of CNS resistance to cloxacillin was noted. Ceftazidime (a third generation cephalosporin) and Ciprofloxacin should preferably not be used in the treatment of patients with DNIs caused by *Acinetobacter baumannii* (ACIBA), as antibiotic resistance patterns approaches that of 57% and 67%, respectively. Colistin is the reported antibiotic of choice in the treatment of patients with DNIs caused by ACIBA with multiple drug resistance.

For microbes with recorded incidences of sensitivity to a specific antibiotic without recorded incidences of antibiotic resistance, a 100% microbial antibiotic sensitivity was inferred. For microbes with recorded incidences of resistance to a specific antibiotic without recorded incidences of antibiotic sensitivity, a 100% microbial antibiotic resistance was however, as a bias, not inferred in this study. This highlights

the need for standardisation of antibiotic susceptibility and resistance profile test batteries for commonly encountered microbes within a population such as those of this study. This would also aid surveillance for multi drug resistant microbes.

It is, however, suggested from this study that the empiric antibiotics used as part of the management of adult patients with DNIs at CHBAH should idealistically include those that are effective against the most frequently isolated microorganisms, namely - *Acinetobacter baumannii* (ACIBA), *Streptococcus pyogenes* (STRPY), *Streptococcus anginosus* (STRAN), *Enterococcus faecalis / Enterococcus faecium* (ENTFA/ENCFE); *Coagulase negative staphylococcocus* (CNS), *Streptococcus Group F* (STRGF), *Streptococcus milleri* (STRMI) and *Klebsiella* (KLESP); respectively.

In practice, this may not be feasible: ACIBA accounted for 53% (n=48) of reported cases of antibiotic resistance, including multiple drug resistance and one cannot justify the use of colistin as a first line empiric antibiotic in patients presenting with DNIs, without evidence based practice guidelines. The reported number of cases of STRPY sensitivity to penicillin/ampicillin was 83% (n=5), and to clindamycin was 17% (n=1). Notably, one patient cultured STRPY as a polymicrobial isolate; one of three microorganisms - with *Staphylococcus haemolyticus* (STAHA) being reported as resistant to penicillin/ampicillin and sensitive to vancomycin (Appendix 1B).

Although empiric antibiotic usage recommendations cannot be based on case reports, principles of usage and associated clinical effectiveness should take into consideration polymicrobial patterns of growth of frequently isolated microbes.

The reported number of cases of STRAN sensitivity to penicillin/ampicillin was 100% (n=4). However, in 3 patients, Gram negative bacilli (GNB) and additionally in 1 of the above 3 patients, Gram positive bacilli (GPB), were observed on microscopy and not identified on culture as polymicrobial isolates with *Streptococcus anginosus* (STRAN) (Appendix 1B).

The reported number of cases of *Enterococcus faecalis* / *Enterococcus faecium* (ENTFA/ENCFE) sensitivity to penicillin/ampicillin was 25% (n=1), and to vancomycin was 75% (n=3) with associated reported resistance to ampicillin/amoxicillin (Appendix 1B). For the one reported case of ENTFA sensitivity to penicillin/ampicillin, polymicrobial growth with *Acinetobacter baumannii* (ACIBA) reported as being resistant to piperacillin-tazobactam, carbapenems and cefepime, a fourth generation cephalosporin, was noted; thus negating the clinical significance of ENTFA sensitivity to penicillin/ampicillin. For the 3 reported cases of ENCFE sensitivity to vancomycin with associated ampicillin/amoxicillin resistance, polymicrobial growth patterns with *Coagulase negative staphylococcocus* (CNS); *Staphylococcus epidermidis* (STAEP) and an unidentified *Bacillus* species (BACSP); and Gram negative bacilli (GNB) on microscopy only, respectively; was also noted. Similarly, these other identified microorganisms in polymicrobial growth patterns with ENCFE, were reported as being vancomycin sensitive with associated resistance to penicillin/ampicillin, and ampicillin/amoxicillin amongst other antibiotics, respectively (Appendix 1B).

CNS and *Streptococcus Group F* (STRGF), isolated as single microorganisms, were reported as being penicillin/ampicillin sensitive. When isolated in a polymicrobial growth pattern with STRGF, anaerobic prevotella, and Gram positive bacilli (GPB) on

microscopy only, CNS and STRGF were both reported as being sensitive to penicillin/ampicillin and vancomycin. The reported number of cases of STRGF sensitivity to penicillin/ampicillin was 60% (n=3) and to clindamycin and vancomycin was 20% (n=1), respectively. Thus, in these instances, isolation of CNS and STRGF as single microorganisms or together in polymicrobial growth patterns, with or without unidentified GPB on microscopy, does not negate their individual sensitivities to penicillin/ampicillin.

The reported number of cases of *Streptococcus milleri* (STRMI) sensitivity to penicillin/ampicillin was 75% (n=3) and to cefotaxime/ceftriaxone (3rd generation cephalosporins) was 25% (n=1), respectively. Polymicrobial isolation of STRMI with unidentified gram negative bacilli (GNB), anaerobic prevotella and unidentified gram positive bacilli (GPB), was noted. However, no reported antibiotic susceptibility profiles for these other microorganisms were documented (Appendix 1B). Thus, the clinical relevance of STRMI sensitivity to penicillin/ampicillin and cefotaxime/ceftriaxone, in this context of polymicrobial isolation, cannot be inferred.

The reported number of cases of *Klebsiella* (KLESP) sensitivity to amoxicillin - clavulanic acid was 40% (n=2), to cefotaxime/ceftriaxone (3rd generation cephalosporins) was 40% (n=2) and to ertapenem was 20%, respectively. KLESP isolated as a single microorganism was sensitive to amoxicillin – clavulanic acid and cefotaxime/ceftriaxone with resistance to ampicillin/amoxicillin, in 2 reported cases. Extended spectrum beta lactamase producing *Klebsiella* (ESBL KLESP) isolated in a polymicrobial growth pattern with a multiple drug resistant *Acinetobacter baumannii* (ACIBA), and *Streptococcus viridans* (STRVI); was reported as being sensitive to

ertapenem with associated resistance to multiple antibiotics including amoxicillin – clavulanic acid and cefotaxime/ceftriaxone, nonetheless. Recommended antibiotic usage entailed escalated dual therapy consisting of a combination of colistin and ceftriaxone for the treatment of all 3 microorganisms (Appendix 1B).

Polymicrobial growth patterns of commonly isolated microorganisms have proven to be incidentally random, unpredictable, complex occurrences; probably largely dependant on host and environmental factors Taking this into consideration when recommending empiric antibiotic usage guidelines, as part of the management of patients with DNIs, could possibly be a challenge of mammoth proportions.

Table 3.22 An overview of reported number of cases of microbial antibiotic resistance

Antibiotics	**No. of cases.**
Cephalosporins 1st generation: Cefazolin 2nd generation: Cefuroxime 3rd generation: Ceftazidime, Cefotaxime / Ceftriaxone 4th generation:Cefepime	18:- (2) (2) (7) (7)
Macrolides (Erythromycin / Azithromycin)	1
Lincosamides (Clindamycin)	1
Sulphonamides (Trimethoprim – Sulphamethoxazole)	7
Carbapenems (Imipenem, Meropenem)	15
Aminoglycosides (Gentamycin, Tobramycin, Amikacin)	8
Quinolones (Ciprofloxacin)	4
Penicillins (Penicillin/Ampicillin; cloxacillin; Ampicillin/amoxicillin; Piperacillin / Tazobactam; Amoxicillin – Clavulanic acid.)	36
Glycopeptides (Vancomycin)	0
Total	90

The reported number of cases of microbial resistance to penicillins in general was 40% (n=36) with an 8% (n=7) reported resistance to amoxicillin-clavulanic acid specifically. In descending order of frequency, the reported number of cases of

microbial resistance to penicillins was of the first order, followed by cephalosporins, third and fourth generations in particular, and carbapenems, respectively.

3.3.1 Profiles of microbial susceptibility and resistance to amoxicillin – clavulanic acid

Microbial sensitivity to amoxicillin – clavulanic acid was reported in only 3 of the 52 patients (6%) and that of microbial resistance in only 5 patients (10%) of the total study population.

3.3.1.1 Profile of microbial susceptibility to amoxicillin – clavulanic acid

1.

Code no.	**Aerobic**	**Anaerobic**	**Other**	**Antibiotic (Sensitivity)**	**Antibiotic (Resistance)**
29	Klebsiella	_	_	amoxicillin-clavulanic acid; cefotaxime/ ceftriaxone	Ampicillin/ amoxicillin
30	_ENTFA _ACIBA	_	_	_ Penicillin/ ampicillin _Ceftazidime	_ _piperacillin-tazobactam; cefepime; imipenem; meropenem

2.

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
42	KLESP	_	_	amoxicillin-clavulanic acid; cefotaxime/ ceftriaxone	Ampicillin/ amoxicillin
43	ACIBA	_	_	Colistin	_piperacillin/ tazobactam; ciprofloxacin; ceftazidime; cefepime; gentamicin; imipenem; meropenem
44	ACIBA	_	_	Colistin	_piperacillin/ tazobactam; trimethoprim-sulfamethoxazole; ciprofloxacin; ceftazidime; cefepime; gentamicin; tobramycin; imipenem; meropenem

3.

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
49	ESCCO	–	–	amoxicillin-clavulanic acid	–

Klebsiella (KLESP) and *Escherichia coli* (ESCCO), both gram negative bacilli (GNB), were the only two of twenty six identified microbes (8%) in this study with reported sensitivity to amoxicillin – clavulanic acid; 3 of 57 microbes (5%) when all pathogens were considered. KLESP, isolated in polymicrobial growth patterns with *Acinetobacter baumannii* (ACIBA) in particular, offset the significance of its sensitivity to amoxicillin – clavulanic acid, with third generation cephalosporins and polymyxins suggested as alternative or combination antimicrobial management options respectively.

In the context of polymicrobial growth patterns with KLESP, ACIBA was reported as conferring resistance to multiple drugs. Specific testing and reporting of ACIBA susceptibility to amoxicillin – clavulanic acid, however, was not documented in this study population. ESCCO was isolated in a monomicrobial, as opposed to polymicrobial, growth pattern, thus its antimicrobial sensitivity pattern was not clinically negated.

3.3.1.2 Profile of microbial resistance to amoxicillin – clavulanic acid

1.

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
56	_ACIBA _ ESBL KLESP _STRVI	_	_	_colistin _ertapenem _cefotaxime/ ceftriaxone (colistin & ceftriaxone combination for all 3 microorganisms)	_trimethoprim-sulfamethoxazole; ciprofloxacin; ceftazidime; cefepime; gentamicin piperacillin/ tazobactam; imipenem; meropenem _ trimethoprim-sulfamethoxazole;ampicillin/ amoxicillin; amoxicillin-clavulanic acid;cefazolin; cefuroxime; cefotaxime/ ceftriaxone; ceftazidime; cefepime _

2.

Code no.	**Aerobic**	**Anaerobic**	**Other**	**Antibiotic (Sensitivity)**	**Antibiotic (Resistance)**
59	_SERSP _ENTCL	_	_	_piperacillin/ tazobactam cefepime _piperacillin/ tazobactam; cefepime	_ampicllin/ amoxicillin _ amoxicillin- clavulanic acid;ampicillin/ amoxicillin

3.

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
61	STRAN	_	GPB 1+ GNB 3+	Penicillin	_
62	_ENTCL _ACIBA _SERMA	_	_	_ciprofloxacin; cefepime; ertapenem _ciprofloxacin _ciprofloxacin; cefepime; ertapenem	_amoxicillin-clavulanic acid;ampicillin/ amoxicillin _piperacillin/ tazobactam; trimethoprim-sulfamethoxazole; meropenem _amoxicillin-clavulanic acid;ampicillin/ amoxicillin
63	ACIBA	_	_	Ciprofloxacin;ceftazidime	Piperacillin/ tazobactam ; meropenem; imipenem

4.

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
66	_	_	GPC 3+	_	_
67	_CITFR _ACIBA	_	_	_Ertapenem _Ceftazidime	_amoxicillin-clavulanic acid;ampicillin/ amoxicillin;cefuroxime; ceftazidime _piperacillin/ tazobactam ; meropenem;cefepime; imipenem

5.

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
69	MOGMO	_	GPC 3+ (lost viability)	Ertapenem; cefepime	amoxicillin-clavulanic acid; trimethoprim-sulfamethoxazole; ampicillin/ amoxicillin; cefazolin; gentamicin
70	MOGMO	_	GPC 3+ (lost viability)	piperacillin/ tazobactam	amoxicillin-clavulanic acid;ampicillin/amoxicillin

Extended spectrum beta lactamase producing *Klebsiella* (ESBL KLESP), *Enterobacter cloacae* (ENTCL), *Serratia marcescens* (SERMA), *Citrobacter freundii* (CITFR) and *Morganella morganii* (MOGMO), interestingly all gram negative bacilli (GNB), were the 5 of 26 identified microbes (19%) in this study with reported resistance to amoxicillin – clavulanic acid, 7 of 57 microbes (12%) when all pathogens were considered.

Recommended alternative antimicrobial coverage included: Ertapenem effective against MOGMO, CITFR, ENTCL, SERMA and ESBL KLESP; cefepime effective

against MOGMO, ENTCL and SERMA ; piperacillin – tazobactam effective against MOGMO and ENTCL and Ciprofloxacin was effective against ENTCL and SERMA.

ESBL KLESP, isolated in a polymicrobial growth pattern with *Acinetobacter baumannii* (ACIBA) in particular, was reported as being resistant to amoxicillin – clavulanic acid, with third generation cephalosporins and polymyxins suggested as an alternative combination antimicrobial management option. This highlights the significance of polymicrobial growth patterns when selecting empiric antimicrobial agents in the management of DNIs. Further research is required to determine if there is a need to define criteria for escalating empiric antibiotic therapy to include combination antimicrobial options in the context of polymicrobial growth patterns, emphasizing co – existing gram negative bacilli (GNB), in DNIs.

Further, *Enterobacter cloacae* (ENTCL), isolated in a polymicrobial growth pattern with *Serratia* species (SERSP), was reported as being resistant to amoxicillin – clavulanic acid; with another B lactamase resistant penicillin, namely piperacillin/tazobactam, or a fourth generation cephalosporin, suggested as an alternative. Both these co – existing GNB displayed antimicrobial susceptibility to the same antimicrobial agents. However, when these two microbial species were isolated in a polymicrobial growth pattern together with *Acinetobacter baumannii* (ACIBA), both still displayed resistance to amoxicillin – clavulanic acid, however the alternative antimicrobial agent of choice in this instance, was a fluoroquinolone, namely ciprofloxacin.

Yet when *Citrobacter freundii* (CITFR) was isolated in a polymicrobial growth pattern with ACIBA, CITFR was resistant to amoxicillin – clavulanic acid, however both microbes displayed differing antimicrobial susceptibility profiles, and a combination of a third generation cephalosporin, namely ceftazidime, and a carbapenem, ertapenem, was suggested as appropriate alternative antimicrobial agents. *Morganella morganii* (MOGMO), isolated in a monomicrobial growth pattern, was susceptible to multiple antibiotic classes, including other beta - lactamase resistant penicillins, carbapenems and fourth generation cephalosporins, as alternatives to amoxicillin – clavulanic acid.

Table 3.23 Amoxicillin – clavulanic acid sensitivity trend for microorganisms

Year No.	Duration	KLESP	ESCCO
1	07/2008-06/2009	_	_
2	07/2009-06/2010	_	_
3	07/2010-06/2011	_	_
4	07/2011-06/2012	_	_
5	07/2012-06/2013	1 (100%)	_
6	07/2013-06/2014	1 (100%)	_
7	07/2014-06/2015	_	1 (100%)

Table 3.24 Amoxicillin – clavulanic acid resistance trend for microorganisms

Year No.	Duration	KLESP	ENTCL	SERMA	CITFR	MOGMO
1	07/2008-06/2009	_	_	_	_	_
2	07/2009-06/2010	_	_	_	_	_
3	07/2010-06/2011	_	_	_	_	_
4	07/2011-06/2012	_	_	_	_	_
5	07/2012-06/2013	_	_	_	_	_
6	07/2013-06/2014	_	_	_	_	_
7	07/2014-06/2015	1	2	1	1	2

The reported sensitivity trend of microorganisms – specifically *Klebsiella* (KLESP) and *Escherichia coli* (ESCCO) - to amoxicillin – clavulanic acid has been fairly consistent over the seven year study period, with per annual, 100% sensitivity inferred. Extended spectrum beta lactamase producing *Klebsiella* (ESBL KLESP), *Enterobacter cloacae* (ENTCL), *Serratia marcescens* (SERMA), *Citrobacter freundii* (CITFR) and *Morganella morganii* (MOGMO), were the 5 of 26 identified microbial subtypes (19%), resistant to amoxicillin – clavulanic acid, 7 of 57 microbes (12%), when all microorganisms were considered. These microorganisms were all reported as being resistant to amoxicillin – clavulanic acid, specifically over the final year of the study period, 07/2014 – 06/2015, with slightly higher incidences reported in ENTCL and MOGMO.

In contrast, no such resistance to amoxicillin – clavulanic acid in any microorganism regardless, was reported over the entire initial 6 years of the study period, 07/2008 – 06/2014. Neither, was there a comparative reported amoxicillin – clavulanic acid

sensitivity profile recorded for the above 5 microorganisms, in the final year of the study. Notably concerning was the ratio of reported amoxicillin – clavulanic acid resistance : sensitivity of 7:1 in the final year of the study; and that this antimicrobial resistance profile was specific to the above mentioned identified gram negative bacilli (GNB) on patient specimen cultures. These micro – organisms were not amongst those most frequently isolated and identified in adult patients with DNIs at CHBAH.

It is however emphasized that profiles of microbial sensitivity and resistance to amoxicillin – clavulanic acid was reported in only 15% (n = 8) of patients with DNIs. Thus, the significance of this reported increased resistance to amoxicillin – clavulanic acid cannot be ascertained due to the small numbers of the study population tested. Furthermore, recommendations for, nor against the continuing use of amoxicillin – clavulanic acid, as the empiric antibiotic of choice, as part of the management of patients with DNIs, cannot be made based on this study.

In this study, when microbial resistance to amoxicillin – clavulanic acid was encountered, suggested alternative antimicrobial agents included third or fourth generation cephalosporins, polymyxins or carbapenems ; either as single agents or as combination therapy. Flouroquinolones and other beta – lactamase resistant penicillins were also favoured alternatives. Recommended alternative antimicrobial coverage, in patients with reported resistance to amoxicillin – clavulanic acid, included: Ertapenem effective against *Morganella morganii* (MOGMO), *Citrobacter freundii* (CITFR), *Enterobacter cloacae* (ENTCL), *Serratia marcescens* (SERMA) and extended spectrum beta lactamase producing *Klebsiella* (ESBL KLESP); cefepime effective against MOGMO, ENTCL and SERMA; piperacillin – tazobactam effective

against MOGMO and ENTCL and Ciprofloxacin was effective against ENTCL and SERMA (Appendix IB).

In a study of 101 patients with deep neck abscesses, amoxicillin - clavulanic acid was used empirically in 82.1% of cases and was based on the coverage of bacteria commonly found in their environment; other recommendations included cefuroxime, meropenem or imipenem; in combination with an antibiotic, which is highly effective against most anaerobes (such as metronidazole or clindamycin).[31] This recommendation for the usage of clindamycin is not in keeping with other literature; this study of 365 patients stated that the most frequently provided treatment regimens, alone or in combination, were amoxicillin - clavulanic acid (59%), second and third generation cephalosporins (37%), ampicillin – sulbactam (13%), clindamycin (11%), metronidazole (4%) and vancomycin (2%).[1]

In this study, when all micro-organisms were considered, only 7% (n=4) were isolated and identified anaerobes; there was no routine reporting on antibiotic susceptibility of identified anaerobes, although metronidazole was suggested for the treatment of prevotella (Appendix 1B). *Staphylococcus epidermidis* (STAEP) resistance to clindamycin was reported in 1% of cases. *Streptococcus pyogenes* (STRPY) and *Streptococcus Group F* (STRGF) sensitivity to clindamycin was reported in 1% of cases, respectively. Routine surveillance of endemic microorganisms and antibiotic susceptibility trends is emphasised.

3.4 Analysis of Histology Distribution

A total of ten patients who had more than one specimen MC&S result per surgical procedure for DNI, did not have additional specimen histological analysis done, and were thus depicted with " - " in Appendix IA. This was also depicted for the one rejected MC&S specimen in Appendix IA.

Table 3.25 Histology distribution: Categorisation of patients' specimen results

Result category	**No. of patient specimens**	**%**
Histology	17	29
No Results	39	67
Rejected	2	4
Total	**58**	**100**

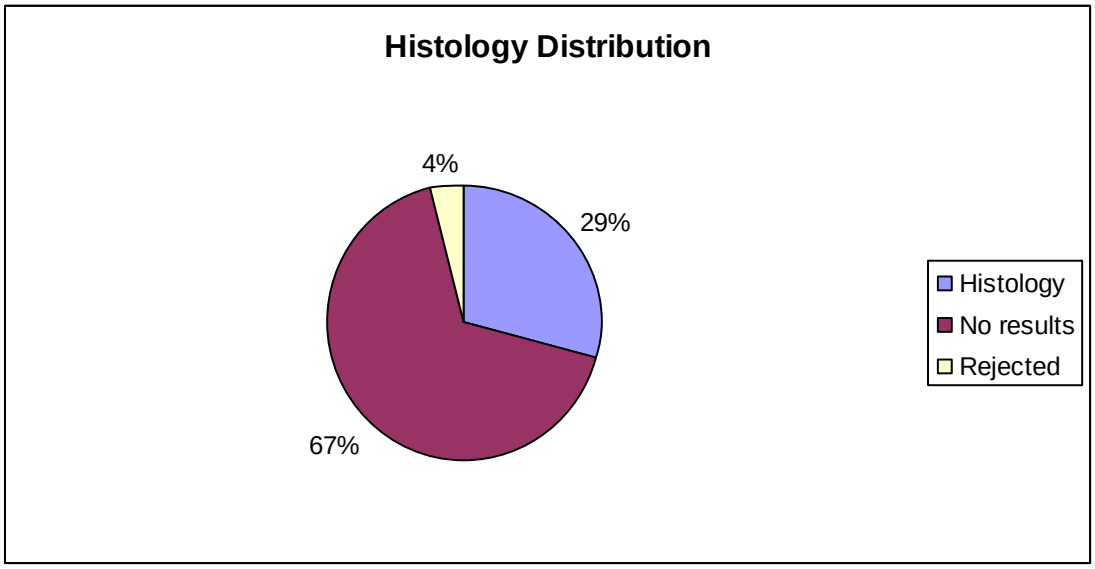

Figure 3.7 Histology distribution: Categorisation of patients' specimen results

A histological analysis was done on 29% (n=17) of patient specimens. For 67% (n=39) of patient specimens, no results were found and for 4% (n=2) of patient specimens, histology results were rejected. The Student's t-test for age and gender distribution per histology distribution, revealed a significant difference ($p<0.05$) between the mean ages of male and female patients, respectively.

3.4.1 Analysis of tissue necrosis distribution

Table 3.26 Tissue necrosis distribution: Categorisation of patients' specimen results

Result category	No. of patient specimens	%
Positive	15	88%
Negative	2	12%
Total	**17**	**100**

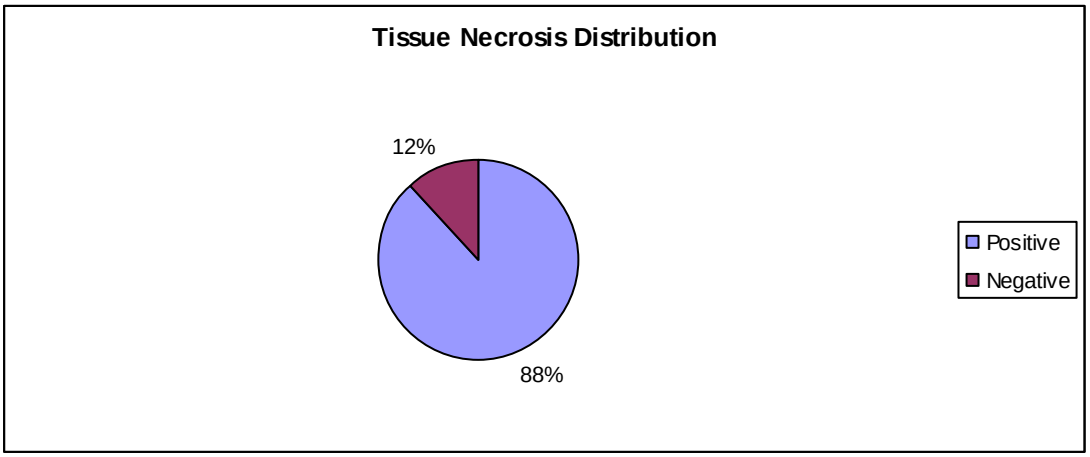

Figure 3.8 Tissue necrosis distribution: Categorisation of patients' specimen results

Tissue necrosis was evident in 88% (n=15) of patients' histological specimens and in 12% (n=2), no tissue necrosis was found. The Student's t-test for age and gender distribution per tissue necrosis distribution, respectively; revealed a significant difference ($p<0.05$).

3.5 Microbiology of DNI and Pulmonary and Extra - Pulmonary Tuberculosis: Case Reports

Key global priorities for tuberculosis (TB) care and control include improving case-detection and detecting cases earlier, including cases of smear-negative disease which are often associated with co-infection with the human immunodeficiency virus (HIV) and with young age. In 2014, an estimated 1.2 million (13%) of the 9.6 million people who developed TB worldwide were HIV-positive. The African Region accounted for 73% of the estimated number of HIV-positive incident TB cases.[29]

1.

Code No.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
38	2013/10/26	M	42	N	N	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
38	_	_	_	_	_

A 42 year old male, retroviral disease positive on anti – retroviral therapy. He had a clinical history of pulmonary TB supported by a miliary TB pattern on pulmonary CT imaging. He presented with a 10 day history of a DNI involving the supraclavicular region, with signs of superimposed acute inflammation. His DNI specimen MC&S result showed no microbial growth; kinyoun stain was negative for acid fast bacilli. There was no record of initiation of empiric anti-TB therapy. The patient was lost to follow up.

2.

Code No.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
26	2012/12/23	M	36	Y	N	_
27	2012/12/23	M	36	N	_	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
26	AFB&MTB (on PCR)	–	–	INH/rifampicin	–
27	_	_	_	_	_

A 36 year old male, retroviral disease positive, CD4 < 350 and not on anti – retroviral therapy. He presented with multiple, bilateral necrotic cervical lymphadenopathy with retropharyngeal abscess formation on CT (duration of associated clinical symptoms of > 1 month). A neck incision & drainage was done for pressure relief on the airway. Intraoperatively, caseous material was drained, thus empiric anti TB treatment was initiated post operatively. His DNI specimen MC&S result showed no microbial growth. Microscopy (fluorochrome method) was negative for acid fast bacilli. Culture was, however, positive for AFB after 14 days incubation. PCR on culture was positive for MTB complex which was INH & Rifampicin susceptible. Sputum TB microscopy (auramine stain) was smear positive for AFB.

3.

Code No.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
6	2009/02/26	F	29	N	N	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
6	_	_	_	_	_

A 29 year old female, retroviral disease positive (AIDS), CD4 159, not on anti – retroviral therapy. She presented with a history of a right submandibular abscess of 6 months duration with signs of superimposed acute inflammation.

Pulmonary TB was diagnosed (smear positive for AFB) and anti – TB treatment was initiated 1 day prior to incision & drainage of DNI. DNI specimen MC&S result (2009/02/26) showed no microbial growth. DNI specimen TB investigation was not recorded on the NHLS data system. On retrospective review, the patient was smear positive for AFB on sputum specimen (11/07/2010) & positive for AFB on blood culture (08/07/2010) after 40 days incubation, despite being on re - treatment for pulmonary TB since May 2010. There was no record on the NHLS data system regarding exclusion of multi drug resistant TB. The patient was diagnosed with AIDS, CD4 174, on anti – retroviral therapy (11/07/2010). She subsequently demised on 22/07/2010.

4.

Code No.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
1	2008/07/21	F	21	Y	N (PNS)	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
1	CANAL *(subculture)*	–	GPC +	–	–

A 21 year old female, retroviral disease positive, CD4 157, not on anti – retroviral therapy. She presented with multiple neck abscesses on CT, predominantly involving the left parapharyngeal space extending to levels III & IV clinically, of two months duration with superimposed signs of acute inflammation. Empiric anti TB treatment was initiated (10/07/2008).

Incision & drainage of DNI (21/07/2008) was done. DNI specimen MC&S result (26/02/2009) showed *Candida albicans* (CANAL) on subculture & gram positive cocci (GPC) on microscopy only. Intra – operative DNI specimen TB investigation results were not recorded on the NHLS data system.

Although pre operative DNI specimen MC&S results were excluded, pre operative DNI aspirate specimen TB investigation results (09/07/08) deserves mention in retrospect: Microscopy was negative for AFB. Culture was positive for AFB after 17 days incubation. Antibiotic susceptibility results showed rifampicin & ethambutol sensitivity; isoniazid & streptomycin resistance.

Of note, a second pre operative DNI aspirate specimen was submitted for TB investigation (09/07/08): Microscopy was negative for AFB. Culture was negative for AFB after 42 days incubation. However, histology of an adenoidal - like post nasal space mass biopsy taken in theatre confirmed mycobacterial infection (necrotizing granulomatous inflammation, positive for AFB on Ziehl Neelsen stain).

Table 3.27 TB investigations in DNIs

TB Investigation	Positive (%)	Negative (%)	Total (%)
Microscopy	**0 (0 %)**	**54 (72%)**	**54 (70%)**
Culture	**1 (50%)**	**21 (28%)**	**22 (29%)**
PCR	**1 (50%)**	**0 (0%)**	**1 (1%)**
Total	**2 (100%)**	**75 (100%)**	**77 (100%)**

Table 3.28 TB investigations in DNIs: Histology & staining

TB Histology & staining	Positive (%)	Negative (%)	Total (%)
ZN stain	**0 (0%)**	**13 (48%)**	**13 (48%)**
Granulomatous inflammation	**0 (0%)**	**14 (52%)**	**14 (52%)**
Total	**0 (0%)**	**27 (100%)**	**27 (100%)**

Notably, peri – operative DNI specimens/aspirates sent for TB investigations, were commented on. There were 5 specimens sent for TB cultures which were contaminated, another specimen was reincubated, however, no result was found. There were 2 specimens not processed due to laboratory errors. TB microscopy was done using the kinyoun, flouorochrome and auramine o staining techniques. TB culture results were reported after a minimum of 29 days and on average after 42 days of incubation.

In patients with extrapulmonary TB DNIs suggested clinically and or radiologically; no growth was observed on culture in 75%, no AFBs detected on microscopy in 50%; AFB positive on culture and mycobacterium complex detected on PCR in 25%.

Therapeutic and empiric anti-TB therapy was initiated in 25% and 50% of patients, respectively.

Pulmonary & extrapulmonary TB could represent sources of TB DNI; or in addition to retroviral disease, it could represent an underlying immune – compromising state that predisposes the host to other opportunistic coexisting microbial DNIs. Although controversial, one extrapulmonary source of cervical tuberculous lymphadenopathy, is the tonsillar crypts; with lymphadenopathy occurring 6 to 9 months therafter. Similarly, in this study, the adenoids were cited as a probable source of TB DNI. There is also a reported association between tuberculous lymphadenitis and HIV infection; thereby facilitating early haematogenous dissemination to single or multiple non – pulmonary sites.[30]

Extrapolations and commonalities amongst patients with suspected or confirmed TB DNIs, in this study, included : Relatively young ages of presentation, an average of 32 years of age. Retroviral disease positivity, relatively low CD4 counts, with patients predominantly not on ARV's. There were associated confirmed microbiological or radiological evidence of pulmonary TB; one exception being an extrapulmonary site (post nasal space), other than TB DNI. Presentations included prolonged duration of symptoms in suspected TB DNI, ranging from 10 days to 6 months and randomised sites of deep neck space involvement.

Primary indication for surgical intervention in suspected TB DNI included superimposed signs of acute inflammation; one exception being that for relief of airway pressure.

DNI specimen MC&S results were reported predominantly as “no growth”, one exception being fungal growth on subculture with gram positive cocci (GPC) on microscopy. Thus, taking into consideration the clinical and radiological findings in patients with suspected TB DNI, MC&S reports of “no growth” should prompt further investigations for other opportunistic infections, eg. mycobacterial and fungal infections, particularly in young, retroviral disease positive patients with low CD4 counts who are notably ARV therapy naive.

The diagnostic yield of TB investigation in DNI relative to other extrapulmonary & pulmonary sites of infection, is inferred to be lower. Submitting greater than one specimen for TB investigation in TB DNI increases the probability of positive diagnostic yield. Microscopy provided a null diagnostic yield in TB DNI relative to pulmonary & other extrapulmonary sites eg. post nasal space. In contrast, TB culture, PCR and histology revealed positive diagnostic yields, with a turnover time to positive culture ranging from 14 to 40 days.

Given the high percentage of negative TB investigation results relative to confirmed TB in DNIs, it is inferred that routine TB investigations in DNIs, not be requested, unless clinically, radiologically or intra operatively suspected. However, low sensitivity & specificity rates for these TB investigation methods in DNI, cannot be excluded.

WHO's policy recommendations state that lateral flow urine lipoarabinomannan assay (LF-LAM) may be used to assist in the diagnosis of TB in HIV positive adult *in-patients* with signs and symptoms of TB (pulmonary and/or extrapulmonary) who have a CD4 cell count less than or equal to 100 cells/µL, or HIV positive patients who are seriously ill (respiratory rate > 30/min, temperature > 39°C, heart rate > 120/min and unable to walk unaided) regardless of CD4 count or with unknown CD4 count (conditional recommendation; low quality of evidence). LF-LAM is easy to perform, requires minimal biosafety requirements, is inexpensive and allows for rapid diagnosis within approximately 25 minutes, thus facilitating the early initiation of anti-TB treatment and reducing mortality in this patient group.[29]

Empiric anti – TB treatment was initiated in two patients with DNI, in this study:

- Indications included clinical and or radiological features with or without intra operative findings suggestive of TB DNI, particularly in retroviral disease positive patients.
- Notably, one case of resistance to 2 anti TB drugs, namely, isoniazid and streptomycin, was reported on a preoperative DNI aspirated specimen antimicrobial sensitivity testing.

There were 4 of 52 patients (8%) with suspected or confirmed TB DNI. This percentage of patients could possibly be an underscore of the actual number of patients with TB DNI at CHBAH. This represents a bias in the study method in that only patients with DNI treated surgically were considered; whilst TB DNI is a disease entity primarily managed medically. Nevertheless, it is inferred from this study that TB DNI is relatively uncommon; in keeping with the literature.[23] However, in the context

of suspected TB DNI, the indications and recommended empiric antimicrobial agents of choice need to be clarified, to prevent the development of MDR TB and associated morbidity & mortality repercussions.

3.6 Fungal DNI

In 4% of patients, fungal DNIs was confirmed on subculture and histology in 50% of these patients, respectively.

Table 3.29 Fungal investigations in DNIs

Fungal investigation	**Positive (%)**	**Negative (%)**	**Total (%)**
Microscopy	0 (0%)	1 (0.1%)	1 (6%)
Culture	1 (subculture)(50%)	2 (14%)	3 (19%)
Histology: ABPAS/PAS staining	1 (50%)	11(79%)	12 (75%)
Total	**2 (100%)**	**14 (100%)**	**16 (100%)**

It is inferred that fungal DNIs are relatively uncommon as well.

3.7 Microbial MC&S and Related Morbidity and Mortality of Patients with DNIs

For each patient with DNI who demised, data tables were extracted from appendix 1A and 1B, respectively. Details related to the number of surgical procedures, specimen MC&S results, source of DNI, duration of hospital admission and most likely cause of death, were reviewed:

1.

Code No.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
47	***2014/08/14***	***F***	***74***	***Y***	***N***	_
48	2014/08/19	F	74	Y	N	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
47	_ENCFE _STAEP _ BACSP (light growth)	_		_*vancomycin* _*vancomycin*	_ampicillin/ amoxicillin _Penicillin/ ampicillin; cloxacillin; erythromycin/ azithromycin; clindamycin; trimethoprim-sulfamethoxazole
48	CORSP	CORSP	_	_	_

The patient had an initial surgical procedure (14/8/2014) & one relook (19/8/2014). She had a polymicrobial aerobic and anaerobic growth pattern; [*Enterococcus faecium* (ENCFE), *Staphylococcus epidermidis* (STAEP), *Corynebacterium* species (CORSP) & an unidentified *Bacillus* species (BACSP)], identified as one of four pathogens per patient. There was no reported resistance to amoxicillin – clavulanic acid; reported vancomycin sensitivity. Directed antibiotic usage was not commenced due to clinical improvement post surgical drainage of DNI. The source of DNI was odontogenic. The duration of hospital admission was 12/8/2014 – 17/10/2014 (prolonged hospital admission). The most likely cause of death was comorbidity related - DCMO with ejection fraction of 20% and persistent hypotension.

2.

Code No.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
51	2014/09/13	F	42	N	Y	N

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
51	–	–	GPC 2+ GNB 2+	–	–

The patient had an initial surgical procedure (13/09/2014).

No microbial growth was identified on culture. Unidentified gram positive cocci (GPC) and gram negative bacilli (GNB) were isolated on microscopy only. Thus no microbial antibiotic susceptibility testing was done. The source of DNI was most likely odontogenic. Notably, CT features were suggestive of pulmonary TB. The histology of DNI showed no granulomata; modified Ziehl Neelsen stain was negative for AFB. The duration of hospital admission was 13/09/2014 – 14/09/2014. The most likely cause of death was septic shock with a metabolic acidosis and acute kidney injury.

3.

Code No.	**Date of Surgery**	**Gender (M/F)**	**Age (years)**	**Bacterial Growth (Y/N)**	**Histology (Y/N)**	**Tissue Necrosis (Y/N)**
24	2012/07/31	M	30	Y	N	_

Code no.	**Aerobic**	**Anaerobic**	**Other**	**Antibiotic(Sensitivity)**	**Antibiotic (Resistance)**
24	STAAU	_	_	Cloxacillin	_

The patient had an initial surgical procedure (31/07/2012). Monomicrobial aerobic growth of *Staphylococcus aureus* (STAAU) was identified. There was no reported resistance to amoxicillin – clavulanic acid; reported cloxacillin sensitivity. The source of DNI was idiopathic. Notably, this patient was newly diagnosed retroviral disease positive, CD4 171; antiretroviral therapy was initiated during this admission.

The duration of hospital admission was 31/07/2012 – 19/09/2012 (prolonged hospital admission, with withdrawal of intensive care unit management secondary to poor prognosis). The most likely cause of death was sepsis related acute on chronic kidney disease and most likely ischaemic brain injury post cardiac arrest and resuscitation.

4.

Code no.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
52	2014/09/29	M	42	Y	Y	Y

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
52	ENCFE	_	G–B 2+	Vancomycin	Ampicillin/ amoxicillin

The patient had an initial surgical procedure (29/09/2014). Monomicrobial aerobic growth [*Enterococcus faecium* (ENCFE)] was identified on culture with unidentified gram negative bacilli (GNB) isolated on microscopy only. Histology confirmed necrotising fasciitis. There was no reported resistance to amoxicillin – clavulanic acid; reported vancomycin sensitivity. The source of DNI was odontogenic. The duration of hospital admission was 29/09/2014 – 30/09/2014. The most likely cause of death was septic shock. The patient demised prior to obtaining microbial MC&S results and thus directed antibiotic usage was never commenced.

5.

Code no.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
42	2014/03/27	M	61	Y	Y	Y
43	2014/03/27	M	61	Y	_	_
44	2014/03/29	M	61	Y	N	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
42	KLESP	_	_	amoxicillin-clavulanic acid; cefotaxime/ ceftriaxone	Ampicillin/ amoxicillin
43	ACIBA	_	_	Colistin	_piperacillin/ tazobactam; ciprofloxacin; ceftazidime; cefepime; gentamicin; imipenem; meropenem
44	ACIBA	_	_	Colistin	_piperacillin/ tazobactam; trimethoprim-sulfamethoxazole; ciprofloxacin; ceftazidime; cefepime; gentamicin; tobramycin; imipenem; meropenem

The patient had an initial surgical procedure (27/03/2014) and one relook surgical procedure (29/03/2014).

There was polymicrobial aerobic growth [*Klebsiella* (KLESP)*, Acinetobacter baumannii* (ACIBA)], identified as one of 2 pathogens per patient. Histology confirmed necrotising fasciitis. There was no reported resistance to amoxicillin – clavulanic acid; reported colistin sensitivity. In the context of multi drug resistance, specific testing of microbial susceptibility to the current empiric antibiotic of choice, amoxicillin – clavulanic acid, is recommended as part of the management of all adult patients with DNI. The source of DNI was odontogenic. The duration of hospital admission was 27/03/2014 – 30/03/2014. The most likely cause of death was sepsis related acute kidney injury (AKI). The patient demised prior to obtaining microbial MC&S results and thus directed antibiotic usage was never commenced. This highlights the need for possible escalation of empiric antibiotic therapy in patients with DNI who present with AKI, however avoidance of nephrotoxic doses is emphasised, notably if colistin or vancomycin, is used.

6.

Code no.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
40	2014/02/26	F	71	N	N (reject)	_
_	2014/02/26	F	71	_ (lab err)	_	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
40	_	_	_	_	_
_	_	_	_	_	_

The patient had an initial surgical procedure (26/02/2014). No microbial growth was identified on culture, thus no microbial antibiotic susceptibility testing was done. The source of DNI was idiopathic. The duration of hospital admission was 25/07/2014 – 26/07/2014. The most likely cause of death was septic shock.

There were 31% (n=16) of patients with “no growth” on DNI cultures and 30% (n=3) of patients with DNIs who demised also had “no growth” on DNI cultures. In elderly patients with idiopathic DNIs, with or without other immune compromising comorbidities, sterile MC&S results should not be taken at face value, especially in the context of empiric antibiotic therapy duration of less than 24 - 48 hours. One cannot rule out opportunistic or atypical bacterial, mycobacterial, fungal or parasitic DNI as causes of morbidity and mortality. In a multi – institutional study of 586 patients with DNIs, culture-confirmed infection of C albicans, was one of the predictors for the development of life-threatening complications.[32]

7.

Code no.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
33	2013/05/16	M	52	N	Y	Y
34	2013/05/20	M	52	Y	N	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
33	_	_	GNB 3+	_	_
34	STEMA	_	_	Cotrimoxazole	_

The patient had an initial surgical procedure (16/05/2013) and a relook surgical procedure (20/05/2013). There was monomicrobial aerobic growth [*Stenotrophomonas maltophilia* (STEMA)] identified on culture with unidentified gram negative bacilli (GNB) isolated on microscopy only. Histology confirmed necrotising fasciitis. There was no reported resistance to amoxicillin – clavulanic acid; reported cotrimoxazole sensitivity. The source of DNI was idiopathic. The duration of hospital admission was 10/05/2013 – 23/05/2013. The most likely cause of death was septic shock.

The patient demised prior to obtaining microbial MC&S results and thus directed antibiotic usage was never commenced. The initial surgical procedure for DNI was on day 6 post hospital admission, as the patient initially responded to medical management including IV amoxillin – clavulanic acid. Subsequently, he developed a necrotizing fasciitis of the neck on day 5 post admission. Thus delays in surgical intervention for DNIs could possibly correlate with increased morbidity & mortality rates, particularly in the elderly.

There were 44% (n=23) of patients with DNIs who had unidentified micro – organisms isolated on microscopy only, without identification on culture; 48% (n=11) of these patients isolated unidentified GNB on microscopy only; 60% (n=6) of patients with DNIs who demised isolated unidentified micro – organisms on microscopy only; an additional patient cultured an unidentified *Bacillus* species (BACSP); 67% (n=4) of these patients isolated unidentified GNB on microscopy only.

The clinical significance of unidentified micro – organisms, including gram negative bacilli (GNB), isolated on microscopy only, and its impact on morbidity and mortality rates in patients with DNI; notably in patients over the age of 40 with or without comorbidities, and in the context of no microbial growth identified on culture, deserves further research.

8.

Code no.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
37	2013/09/22	M	50	N	N	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
37	_	_	GNB 3+	_	_

The patient had an initial surgical procedure (22/09/2013). No microbial growth was identified on culture with unidentified GNB isolated on microscopy only. Necrotising fasciitis of the neck & anterior chest wall was clinically apparent but not confirmed on histology. Thus no microbial antibiotic susceptibility testing was done. The source of DNI was odontogenic. The duration of hospital admission was 20/09/2013 – 24/10/2013. The most likely cause of death was septicaemia. Notably, the patient was on day 3 of colistin for *Acintobacter baumanii* (a GNB) septicaemia confirmed on blood culture. This GNB identified on blood culture was most likely the same unidentified GNB isolated on microscopy of the submitted DNI specimen.

These findings are inconsistent with that of the literature.[3] Apart from the delayed initiation of directed antibiotic treatment in this patient, routine requests for blood cultures, as part of the management of patients with DNIs, cannot be recommended based on these findings alone.

9.

Code no.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
19	2011/06/29	F	54	Y	N (tonsil)	_
20	2011/06/29	F	54	Y	_	_
21	2011/06/29	F	54	Y	_	_
22	2011/06/29	F	54	Y	_	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
19	STAHA	Prevotella	_	Vancomycin (aerobe). Suggested flagyl (anaerobe)	Cloxacillin
20	ACIBA	Prevotella	GPC 3+ GPB 2+	Colistin (aerobe)	gentamicin; ceftazidime; piperacillin-tazobactam; cefepime; amikacin; tobramycin; ciprofloxacin imipenem; meropenem; cotrimoxazole
21	_	Prevotella	GPB 1+	_	_
22	_	Prevotella	_	_	_

The patient had an initial surgical procedure (29/06/2011).

Polymicrobial mixed aerobic and anaerobic growth [*Staphylococcus haemolyticus* (STAHA), *Acinetobacter baumannii* (ACIBA), Prevotella], was identified as one of 3 pathogens per patient. Unidentified gram positive cocci (GPC) and gram positive bacilli (GPB) were isolated on microscopy only. This patient had 4 specimens submitted to the NHLS laboratory thus increasing the yield of microbes isolated and identified.

There was no reported resistance to amoxicillin – clavulanic acid; reported vancomycin & colistin sensitivity. Notably, this patient clinically improved post surgical intervention for DNI and thus directed antibiotic therapy was not commenced. In this context of surgical intervention for DNI, with curative intent and that most DNIs are polymicrobial in nature, the role of diagnostic surgical intervention with intra - operative specimens submitted for MC&S analysis, can then be argued to be debatable, unless signs and symptoms of septicaemia are already evident or eminent.

No routine antibiotic susceptibility testing was done for anaerobic micro-organisms. The source of DNI was tonsillitis. The duration of hospital admission was 29/06/2011 – 25/08/2011. The most likely cause of death was nosocomial acquired pneumonia related sepsis, possibly secondary to a post tracheostomy iatrogenic trachea – oesophageal fistula.

10.

Code no.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
7	2009/04/14	M	23	Y	Y	Y
8	2009/04/14	M	23	Y	_	_

Code no.	Aerobic	Anaerobic	Other	Antibiotic(Sensitivity)	Antibiotic (Resistance)
7	_STRGF _CNS	Prevotella	GPB 2+	_Penicillin/ ampicillin; vancomycin _Penicillin/ ampicillin; vancomycin (aerobes)	–
8	STRMI	Prevotella	GPB 1+	Penicillin	_

The patient had an initial surgical procedure (14/04/2009). Polymicrobial mixed aerobic and anaerobic growth [*Streptococcus Group F* (STRGF), *Coagulase negative staphylococcocus* (CNS), *Streptococcus milleri* (STRMI), Prevotella] was identified as 1 of 4 pathogens per patient. Unidentified gram positive bacilli (GPB) were isolated on microscopy only. This patient had 2 specimens submitted to the NHLS laboratory thus increasing the yield of microbes isolated and identified. Histology confirmed necrotising fasciitis.

There was no reported resistance to amoxicillin – clavulanic acid; reported vancomycin sensitivity. Notably, this patient clinically improved post surgical intervention for DNI and thus directed antibiotic therapy was not commenced. The source of DNI was idiopathic. The duration of hospital admission was 12/04/2009 – 11/05/2009. The patient was discharged from hospital 06/05/2009 and readmitted 10/05/2009.

The most likely cause of death was nosocomial acquired gastroenteritis related sepsis with underlying acute kidney injury (was receiving dialysis) secondary to DNI.

It is noted that 30% (3 /10) of patients with DNIs who demised had either monomicrobial [*Enterococcus faecium* (ENCFE)] or polymicrobial [ENCFE, *Staphylococcus epidermidis* (STAEP) & *Corynebacterium* species (CORSP)][*Staphylococcus haemolyticus* (STAHA), *Acinetobacter baumannii* (ACIBA) & Prevotella] growth patterns with reported vancomycin sensitivity. Recommended usage of vancomycin was as a single agent or in combination with other directed antibiotics, in this instance, colistin. The use of vancomycin & colistin either as single agents, or in combination, as empiric antibiotics in patients with DNIs & whether this will significantly affect mortality rates, needs further research.

3.8 Sources of DNIs in Survivors versus Patients with DNIs who Demised

The sources of DNI in that of survivors, was idiopathic (52%) in most instances, followed by odontogenic origin (26%). In patients who demised, secondary to DNIs, odontogenic sources (60%) was most frequently encountered.[1] There were 32% of survivors of DNI of idiopathic sources who were retroviral disease positive. One survivor of DNI of idiopathic source, was a patient with GPA in remission, secondary to iatrogenic immunosuppressive steroid therapy. Salmonella from idiopathic sources was identified in 4% (n=2) of patients with DNIs; who were interestingly also newly diagnosed with diabetes mellitus type II, in keeping with the literature.[2,24] Thus the role of underlying host immunity, or rather lack therof, in DNIs, including that from idiopathic sources, is emphasized.

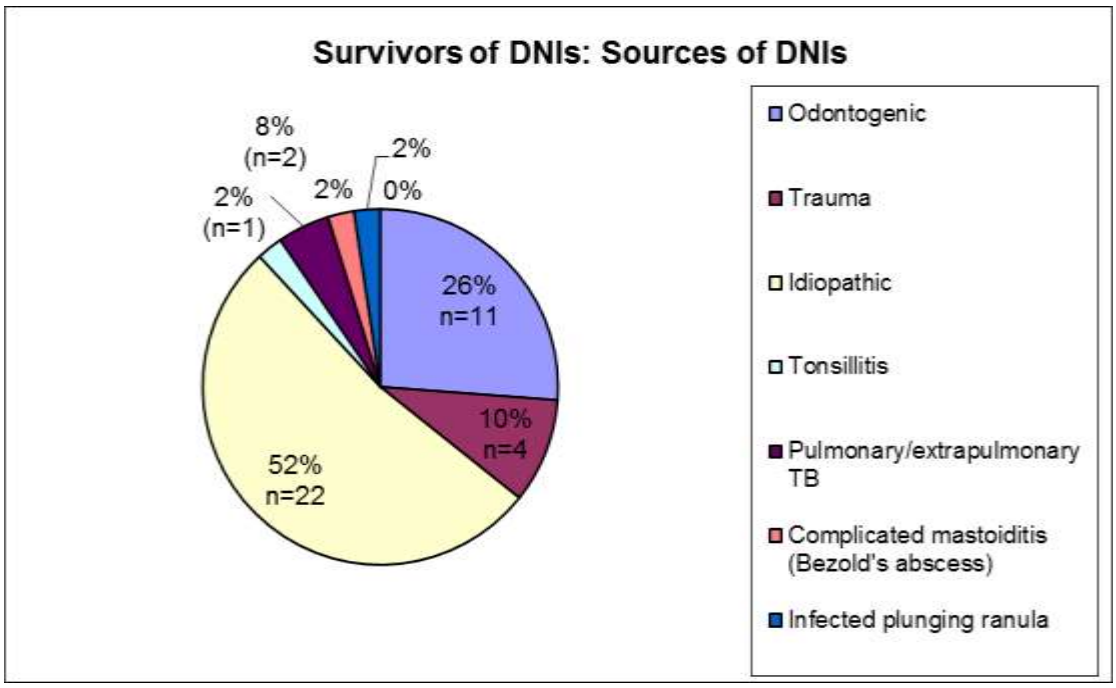

Figure 3.9 Sources of DNIs in Survivors

97

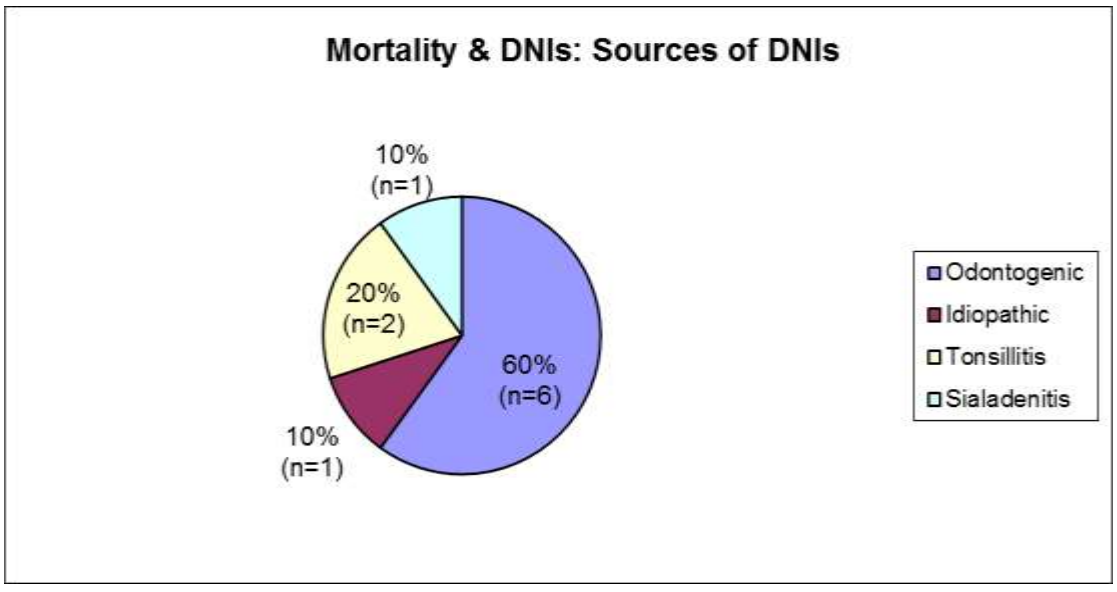

Figure 3.10 Sources of DNIs in patients who demised

4 SUMMARY, CONCLUSION AND RECOMMENDATIONS

4.1 Summary

Admissions of patients with DNIs significantly increased during the latter period of the study (07/2012 – 06/2015). There was a 19% mortality rate, with an odontogenic source of DNI in 60% of patients who demised and in 26% of survivors, respectively. In 52% of survivors, the source of DNI was idiopathic. The distribution of positive cultures was 69% (n=48) and that of negative cultures- implying no growth, was 31% (n=22), from 70 specimens of 52 patients with DNIs. There were 66% (n=23) of patients with positive cultures who had monomicrobial growth identification with polymicrobial growth in 34% (n=12); aerobes only were identified in 89% (n=31) and both aerobes and anaerobes identified in 11% (n=4). There were 92% (n=24) of microorganisms which were aerobic and 8% (n=2) anaerobic. When all pathogens were considered, 60% (n=34) had polymicrobial growth patterns and 40% (n=23) monomicrobial growth; 93% (n= 53) were aerobic and 7% (n=4) anaerobic; 57 microorganisms were identified on culture as 26 different microorganism subtypes in 35 patients with positive cultures.

The distribution of negative cultures in this study, relative to the literature, was not negligible (31% versus 19% respectively); in patients with positive cultures; when all micro-organisms were considered, polymicrobial and aerobic growth patterns were more common than monomicrobial and anaerobic growth identification; whether this represents a true reflection of anaerobes involved in DNIs or an underestimation; requires further clarification.

In descending order, aerobic ACIBA, *Streptococcus pyogenes* (STRPY), *Streptococcus anginosus* (STRAN), *Enterococcus faecalis / Enterococcus faecium* (ENTFA/ENCFE); *Coagulase negative staphylococcocus* (CNS), *Streptococcus Group F* (STRGF), *Streptococcus milleri* (STRMI), *Klebsiella* (KLESP); and anaerobic Prevotella, were the more frequently isolated and identified micro – organisms in adult patients with DNIs at CHBAH. Thus, the most frequently isolated aerobic microorganisms included gram negative bacilli and streptococcus species. Although infrequently encountered; atypical micro – organisms in this patient population included fungi, TB and salmonella.

The study hypothesis is that the empiric antibiotic therapy currently in use, amoxicillin – clavulanic acid, is effective against microbes commonly involved in DNIs in adults at CHBAH. Utilising this report as a retrospective tool to guide empiric antibiotic usage; the small study population number and that suggestions are primarily based on inferences only; is emphasized: In retrospective review, this hypothesis cannot be proved nor disproved: It is suggested from this study that the empiric antibiotics used as part of the management of adult patients with DNIs at CHBAH should idealistically include those that are effective against the most frequently isolated microorganisms, although this might not always be feasible, nor practical.

Notably concerning was the ratio of reported amoxicillin – clavulanic acid resistance : sensitivity of 7:1 in the final year of the study (07/2014 – 06/2015); and that this antimicrobial resistance profile was specific to identified gram negative bacilli (GNB) on patient specimen cultures: These micro – organisms, namely extended spectrum

beta lactamase producing *Klebsiella* (ESBL KLESP), *Enterobacter cloacae* (ENTCL), *Serratia marcescens* (SERMA), *Citrobacter freundii* (CITFR) and *Morganella morganii* (MOGMO), were not amongst those most frequently isolated and identified in adult patients with DNIs at CHBAH - 5 of 26 identified microbes (19%), resistant to amoxicillin – clavulanic acid; 7 of 57 microbes (12%). The significance of the increase in the reported resistance to amoxicillin – clavulanic acid cannot be ascertained due to the small numbers of the study population tested.

Klebsiella (KLESP) and *Escherichia coli* (ESCCO), both gram negative bacilli (GNB), were the only 2 of 26 identified microbes (8%) with reported sensitivity to amoxicillin – clavulanic acid; 3 of 57 microbes (5%) when all pathogens were considered. Microbial sensitivity to amoxicillin – clavulanic acid was reported in only 3 of the 52 patients (6%) and that of microbial resistance in only 5 patients (10%) of the total study population. It is thus emphasized that profiles of microbial sensitivity and resistance to amoxicillin – clavulanic acid was reported in only 15% (n = 8) of patients with DNIs.

In this study, when microbial resistance to amoxicillin – clavulanic acid was encountered, recommended alternative antimicrobial coverage included: Ertapenem effective against *Morganella morganii* (MOGMO), *Citrobacter freundii* (CITFR), *Enterobacter cloacae* (ENTCL), *Serratia marcescens* (SERMA) and extended spectrum beta - lactamase producing *Klebsiella* ESBL KLESP; cefepime effective against MOGMO, ENTCL and SERMA; piperacillin – tazobactam effective against MOGMO and ENTCL and Ciprofloxacin was effective against ENTCL and SERMA.

Acinetobacter baumannii (ACIBA) accounted for 53% (n=48) of cases of antibiotic resistance with that to Ceftazidime and Ciprofloxacin approached 57% and 67%, respectively. Colistin was effective against ACIBA with reported multiple drug resistance. Amoxicillin – clavulanic acid & Cefotaxime/Ceftriaxone were effective against *Klebsiella* (KLEBS), with equivalent susceptibility and associated resistance profiles of 67% and 33%, respectively. When only reported antimicrobial sensitivity profiles were considered, that of klebsiella to ertapenem was 20%. Ertapenem was effective against polymicrobial growth of ESBL KLESP with multiple drug resistant ACIBA and *Streptococcus viridans* (STRVI); with associated resistance to amoxicillin – clavulanic acid and cefotaxime/ceftriaxone. Recommended alternative antibiotic usage entailed escalated dual therapy consisting of a combination of colistin (a polymyxin) and ceftriaxone for the treatment of all 3 microorganisms.

Penicillin/ampicillin was effective against *Streptococcus anginosus* (STRAN), *Coagulase negative staphylococcocus* (CNS), *Streptococcus Group F* (STRGF), *Streptococcus milleri* (STRMI) and *Streptococcus pyogenes* (STRPY), with a sensitivity of 100%, 67%, 60%, 75% and 83%, respectively; that of STRGF and STRPY to clindamycin was 20% and 17%, respectively; and that of STRMI to cefotaxime/ ceftriaxone was 25% . Vancomycin was effective against polymicrobial growth that included STRPY with *Staphylococcus haemolyticus* (STAHA); and against that of *Enterococcus faecium* (ENTFA/ENCFE) with *Coagulase negative staphylococcocus* (CNS); ENCFE with *Staphylococcus epidermidis* (STAEP) and an unidentified *Bacillus* species (BACSP); ENCFE and Gram negative bacilli (GNB) on microscopy only, respectively; with associated resistance to penicillin/ampicillin. ENTFA/ENCFE sensitivity to vancomycin was 75% (n=3).

TB and fungal DNIs are relatively uncommon: In 8% of patients with DNIs, extrapulmonary TB was suggested clinically and or radiologically; with no growth on culture in 75%, no AFBs detected on microscopy in 50%, AFB positive on culture and mycobacterium complex detected on PCR in 25%. Therapeutic and empiric anti-TB therapy was initiated in 25% and 50% of patients, respectively. In 4% of patients, fungal DNIs was confirmed on subculture and on histology in 50% of these patients, respectively. Further, with regards to atypical micro-organisms isolated in patients with DNIs, Salmonella was identified in 4% of patients who were also newly diagnosed with diabetes mellitus type II.

4.2 Conclusion

Patients with DNIs at CHBAH treated with the combined modalities of surgical intervention and empiric antibiotic usage, currently amoxicillin – clavulanic acid, has resulted in an overall favourable outcome associated with a low mortality rate of 19%. In this study, when microbial resistance to amoxicillin – clavulanic acid was encountered, suggested alternative antimicrobial agents included third or fourth generation cephalosporins, polymyxins or carbapenems; either as single agents or as combination therapy. Flouroquinolones and other beta – lactamase resistant penicillins were also favoured alternatives. However, alternative empiric antibiotic usage cannot be recommended, based on this study.

4.3 Recommendations

The epidemiology of DNIs needs to be monitored for changing trends and the impact of underlying host immunity and developing microbial multi-drug resistance, established. Surveillance at a NHLS level should include mandatory susceptibility testing of all microbes commonly identified in adult DNI MC&S specimens, to the then current loco- regional empiric antibiotics in use. The role of susceptibility testing of microbes not commonly identified in adult DNI MC&S specimens, to the then current loco- regional empiric antibiotics in use, needs further review, in selected patients on a clinical case by case basis. Thus for this study population at CHBAH, the antimicrobial agent test battery should include routine amoxicillin – clavulanic acid susceptibility testing. This also serves as a method of standardization which has reproducibility and can be used in comparative analysis.

A key factor in ensuring adequate empiric antibiotic coverage, would be to elucidate the clinical significance and impact on morbidity and mortality rates, of idiopathic sources of DNIs amongst other sources, unidentified microbes, isolated on microscopy only but not cultured, with particular emphasis on unidentified gram negative bacilli (GNB) , that of “no growth” & polymicrobial growth patterns; including the relevance and cost versus benefit of intra – operative DNI specimen collection in guiding directed antibiotic usage.

Further research must also provide a superior quality of evidence in routinely monitoring & analyzing trends of common micro – organisms implicated in adults with DNIs including that isolated in patients with DNIs who demised and empiric antibiotic

usage patterns in adults with DNIs, with intent to periodically update practice guidelines at a regional and even national health level, thereby deterring multidrug resistance, in part contributing to the management of patients with DNIs. To ensure an adequate patient population, a sequel to this study should possibly include Charlotte Maxeke and Helen Joseph academic hospitals, which are affiliated to the University of the Witwatersrand, in the surveillance program as well.

The recommendation for continued empiric usage of amoxicillin – clavulanic acid as well as that of appropriate alternative empiric antibiotic usage; in this study population, requires further periodic surveillance of commonly identified microbes and antimicrobial sensitivity results; utilizing standardized protocols. The increase in the reported resistance to amoxicillin – clavulanic acid, particularly in uncommonly identified gram negative bacilli (GNB); as well as polymicrobial growth patterns and its influence on antimicrobial sensitivity, also requires further elucidation.

5 REFERENCES

1. Boscolo RP, Stellin M, Muzzi E, Mantovani M, Fuson R, Lupato V, et al. Deep neck space infections: A study of 365 cases highlighting recommendations for management and treatment. Eur Arch Otorhinolaryngol. 2012;269(4):1241–49.
2. Vieira F, Allen SM, Stocks RMS, Thompson JW. Deep neck infection. Otolaryngol. Clin. North Am. 2008;41:459-483.
3. Huang TT, Liu TC, Chen PR, Tseng FY, Yeh TH, Chen YS. Deep neck infection: analysis of 185 cases. Head Neck. 2004;854-860.
4. Caccamese JF, Coletti DP. Deep neck infections: clinical considerations inaggressive disease. Oral Maxillofacial Surg Clin N Am. 2008;20:367-380.
5. Hasegawa J, Hidaka H, Tateda M, Kudo T, Sagai S, Miyazaki M et al. An analysis of clinical risk factors of deep neck infection. Auris Nasus Larynx. 2011;38:101-107.
6. Weed HG, Forest LA. Deep neck infection. In: Cummings CW, Flint PW, Harker LA, et al, editors. Otolaryngology: head and neck surgery. 4th edition. Philadelphia: Elsevier Mosby; 2005. p. 2515 2524.
7. Har-El G, Aroesty J, Shaha A, Lucente F. Changing trends in deep neck abscess: a retrospective study of 110 patients. Oral Surg Oral Med Oral Pathol. 2006;77(5):446-450.
8. Chettiar TP. A prospective comparative study of potential risk factor between Ludwig's Angina and localised odontogenic abscesses [dissertation]. Johannesburg: University of Witwatersrand, 2006.
9. Santos GP, Perez PB, Martin ACM. Deep neck infection: review of 286 cases. Acta Otorrinolaringo Esp. 2012;63:31-41.

10. Motahari SJ, Poormoosa R, Nikkhah M, Bahari M, Shirazy SM. Treatment and prognosis of deep neck infections. Indian J Otolaryngo Head Neck Surg. 2015;67(Suppl 1):134-137.

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

12. Reynolds SC, Chow AW. Life-threatening infections of the peripha-ryngeal and deep fascial spaces of the head and neck. Infect Dis Clin N Am. 2007;21:557-576.

13. Hull MW, Chow AW. Indigenous microflora and innate immunity of the head and neck. Infect Dis Clin N Am. 2007;21:265-285.

14. Marioni G, Staffieri A, Parisi A, Zuccon A, Staffieri C, Sari M et al. Rational diagnostic and therapeutic management of DNI: analysis of 233 consecutive cases. Ann Otol Rhinol Laryngol. 2010;119(3):181-187.

15. Roscoe DL , Hoang L. Microbiological investigations for head and neck infections. Infect Dis Clin N Am. 2007;21:283-304.

16. Sethi DS, Stanley RE. Deep neck abscesses – changing trends. J Laryngol Otol. 1994;108:138-143.

17. Celakovsky P, Kalfert D. Bacteriology of deep neck infections: analysis of 634 patients. Aust Dent J. 2015;2:212-215.

18. Barber BR, Biron VL, Ma A, Seikaly H. Factors associated with deep neck space infections: targeting multiple fronts. J Otolaryngol Head Neck Surg. 2014;43(1):35.

19. Constantinides MRCS, Passant C, Waddell AN. Mycotic pseudoaneurysm of common carotid artery mimicking parapharyngeal abscess. J Laryngol Otol. 2000;114:796-797.

20. Obregon-Guerrero G, Martinez-Ordaz JL, Moreno-Aguilera E, Ramirez-Martinez M, Pena-Garcia JF, Perez-Alvarez C. Deep neck abscess. Factors related to reoperation and mortality. Cir Cir. 2013;81(4):299-306.

21. Laupland KB. Vascular and parameningeal infections of the head and neck. Infect Dis Clin N Am. 2007;577-590.

22. Chi TH, Tsoa YH, Yuan CH. Influence of patient age on DNI: clinical etiology and treatment outcome. Otolaryngol Head Neck Surg. 2014;151(4):586-90.

23. Bakir S, Tanriverdi NH, Gun R, Yorgansilar AE, Yildirim M, Tekbas G et al. Deep neck infections: a retrospective review of 173 cases. Am J Otolaryngol. 2012;33(1):56-63.

24. Tai PW, Hung SH, Huang CH. Neck abscess caused by Salmonella enterica serotype Enteritidis. J. Formos. Med. Assoc. 2013;112:434-435.

25. Brook I. Microbiology and principles of antimicrobial therapy for head and neck infections. Infect Dis Clin N Am. 2007;355-391.

26. Ali A, Kurien M, Mathews SS & Mathews J. Complications of infective rhinosinusitis: experience from a developing country. Singapore Med J. 2005;46:540-544.

27. Butler JC, Hoffman J, Centron MS, Elliot JA, Facklam RR, Breiman RF. The continued emergence of drug resistant Streptococcus pneumoniae in the United States: An update from the Centre of Disease Control and Prevention's Pneumococal Sentinal Surveillance System. J Infect Dis. 1996;174:986-993.

28. Ball CN, Connor-Linton J. Web chi square calculator. www.georgetown.edu/faculty/ballc/webtools/web_chi.html Department of Linguistics, University of Georgetown 2007.

29. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV. Policy guidance. World Health Organization 2015. ISBN 978 92 4 150963

30. Khuzwayo ZB, Naidu TK. Head and neck tuberculosis in KwaZulu –Natal, South Africa. J Laryngol Otol. 2014;128:86-90.

31. Brito TP, Hazboun IM, Fernandes FL, Bento LR, Zappelin CEM, Chone CT, Crespo AN. Deep neck abscesses: study of 101 cases. Braz J Otorhinolaryngol. 2017;83(3):341-348.

32. Mejzlik J, Celakovsky P, Tucek L, Kotulek M, Vrbacky A, Matousek P et al. Univariate and multivariate models for the prediction of life-threatening complications in 586 cases of deep neck space infections: retrospective multi-institutional study. J Laryngol Otol. 2017;131:779–784.

6 APPENDICES

6.1 Appendix IA – Linked Secured File 1

Code No.	Date of Surgery	Gender (M/F)	Age (years)	Bacterial Growth (Y/N)	Histology (Y/N)	Tissue Necrosis (Y/N)
1	2008/07/21	F	21	Y	N (PNS)	_
2	2008/12/11	M	27	N	N	_
3	2008/12/11	M	27	N	_	_
4	2008/12/24	F	21	N	N	_
5	2009/02/20	F	27	Y	Y	N
6	2009/02/26	F	29	N	N	_
7	2009/04/14	M	23	Y	Y	Y
8	2009/04/14	M	23	Y	_	_
9	2010/01/29	M	28	N	Y	Y
10	2010/02/17	M	30	Y	N	_
11	2010/03/28	F	31	Y	N	_
12	2010/04/04	F	28	N	N	_
13	2010/04/04	F	28	N	_	_
14	2011/02/05	M	28	Y	N	_
15	2011/02/21	F	52	Y	N	_
16	2011/03/12	M	34	Y	N	_
17	2011/05/17	M	50	N	N	_
18	2011/06/23	F	57	N	N	_
19	2011/06/29	F	54	Y	N (tonsil)	_
20	2011/06/29	F	54	Y	_	_
21	2011/06/29	F	54	Y	_	_
22	2011/06/29	F	54	Y	_	_
23	2012/04/24	M	35	Y	N	_
24	2012/07/31	M	30	Y	N	_
25	2012/09/13	M	33	N	Y	Y
26	2012/12/23	M	36	Y	N	_
27	2012/12/23	M	36	N	_	_
28	2013/02/26	M	24	Y	Y	Y
29	2013/03/23	F	57	Y	N (mastoid)	_
30	2013/03/23	F	57	Y	_	_
31	2013/04/16	M	36	Y	Y	Y
32	2013/04/16	M	36	N	_	_
33	2013/05/16	M	52	N	Y	Y
34	2013/05/20	M	52	Y	N	_
35	2013/06/13	M	39	Y	N	_

36	2013/07/25	F	19	Y	Y	Y
37	2013/09/22	M	50	N	N	_
38	2013/10/26	M	42	N	N	_
39	2013/12/13	M	53	Y	N	_
40	2014/02/26	F	71	N	N (reject)	_
_	2014/02/26	F	71	_ (lab err)	_	_
41	2014/03/17	F	43	Y	N	_
42	2014/03/27	M	61	Y	Y	Y
43	2014/03/27	M	61	Y	_	_
44	2014/03/29	M	61	Y	N	_
45	2014/05/07	M	50	Y	N	_
46	2014/05/17	M	22	N	N	_
47	2014/08/14	F	74	Y	N	_
48	2014/08/19	F	74	Y	N	_
49	2014/08/21	M	57	Y	Y	Y
50	2014/09/03	F	30	N	N	_
51	2014/09/13	F	42	N	Y	N
52	2014/09/29	M	42	Y	Y	Y
53	2014/10/02	M	35	N	N	_
54	2014/10/02	M	39	Y	N	_
55	2014/10/02	M	39	Y	_	_
56	2014/10/28	F	68	Y	N	_
57	2014/10/30	M	53	Y	N	_
58	2014/10/30	M	20	Y	N	_
59	2014/11/28	M	53	Y	Y	Y
60	2014/12/14	F	45	Y	N	_
61	2014/12/19	M	22	Y	Y	Y
62	2014/12/23	M	22	Y	Y	Y
63	2014/12/23	M	22	Y	_	_
64	2014/12/26	F	64	Y	N	_
65	2015/01/30	M	52	N	N	_
66	2015/02/05	M	39	N	N (lab error)	_
67	2015/02/09	M	39	Y	N	_
68	2015/03/07	M	20	Y	N	_
69	2015/06/21	M	77	Y	Y	Y
70	2015/06/22	M	77	Y	Y	Y

6.2 Appendix IB – Linked Secured File 2

Code no.	Aerobic	Anaerobic	Other	Antibiotic (Sensitivity)	Antibiotic (Resistance)
1	CANAL *(subculture)*	–	GPC +	_	–
2	_	_	GPC 2+	_	_
3	_	_	GNB (mixed enteric)	_	_
4	_	_	_	_	_
5	STRMI	_	GNB 3+	Penicillin	_
6	_	_	_	_	_
7	_STRGF _CNS	Prevotella	GPB 2+	_Penicillin/ ampicillin; vancomycin _Penicillin/ ampicillin; vancomycin (aerobes)	–
8	STRMI	Prevotella	GPB 1+	Penicillin	_
9	_	_	GPC 3+	_	_
10	STRGF	_	–	Penicillin/ ampicillin; clindamycin	–
11	STRMI	Prevotella	GPB 2+	Penicillin (aerobes); cefotaxime/ ceftriaxone	–
12	_	_	_	_	_
13	_	_	_	_	_
14	STRGF	_	GPB 2+	Penicillin/ ampicillin	_
15	Salmonella	_	–	Ceftriaxone/ cefotaxime; ciprofloxacin	–
16	STRVI	_	_	Penicillin	_
17	_	_	GNB 1+	_	_
18	_	_	_	_	_
19	STAHA	Prevotella	_	Vancomycin (aerobe). Suggested flagyl (anaerobe)	Cloxacillin

20	ACIBA	Prevotella	GPC 3+ GPB 2+	Colistin (aerobe)	gentamicin; ceftazidime; piperacillin-tazobactam; cefepime; amikacin; tobramycin; ciprofloxacin imipenem; meropenem; cotrimoxazole
21	_	Prevotella	GPB 1+	_	_
22	_	Prevotella	_	_	_
23	STRPY	_	_	Penicillin/ ampicillin	_
24	STAAU	_	_	Cloxacillin	_
25	_	_	GPC 3+	_	_
26	AFB&MTB (on PCR)	–	_	INH/rifampicin	_
27	_	_	_	_	_
28	STRAN	_	GNB 3+	Penicillin/ ampicillin	_
29	Klebsiella	_	_	amoxicillin-clavulanic acid; cefotaxime/ ceftriaxone	Ampicillin/ amoxicillin
30	_ENTFA _ACIBA	–	_	_ Penicillin/ ampicillin _Ceftazidime	_ _piperacillin-tazobactam; cefepime; imipenem; meropenem
31	CNS	_	_	Penicillin/ ampicillin	_
32	_	_	G+C 3+	_	_
33	_	_	GNB 3+	_	_
34	STEMA	_	_	Cotrimoxazole	_
35	STRAN	_	_	Penicillin/ ampicillin	_
36	GPC (non viable)	–	GPC 3+ GPB 2+	–	–
37	_	–	GNB 3+ GPC 1+	–	–
38	_	_	_	_	_
39	SALSP	_	_	Ceftriaxone/ cefotaxime	_
40	_	_	_	_	_
_	_	_	_	_	_

41	-STAHA -HAEPA -STRPY	_	_	_Vancomycin _Ampicillin/ amoxicillin _Penicillin/ ampicillin	_Penicillin/ ampicillin; cloxacillin – –
42	KLESP	_	_	amoxicillin-clavulanic acid; cefotaxime/ ceftriaxone	Ampicillin/ amoxicillin
43	ACIBA	_	_	Colistin	_piperacillin/ tazobactam; ciprofloxacin; ceftazidime; cefepime; gentamicin; imipenem; meropenem
44	ACIBA	_	_	Colistin	_piperacillin/ tazobactam; trimethoprim-sulfamethoxazole; ciprofloxacin; ceftazidime; cefepime; gentamicin; tobramycin; imipenem; meropenem
45	_ CNS _ENCFE	–	–	_vancomycin _vancomycin	_Penicillin/ ampicillin; cloxacillin _ampicillin/ amoxicillin
46	_	_	GPC 3+	_	_
47		_		_*vancomycin* _*vancomycin*	_ampicillin/ amoxicillin _Penicillin/ ampicillin; cloxacillin; erythromycin/ azithromycin; clindamycin; trimethoprim-sulfamethoxazole

	_ENCFE _STAEP _ BACSP (light growth)				
48	CORSP	CORSP	_	_	_
49	ESCCO	_	_	amoxicillin-clavulanic acid	_
50	_	_	_	_	_
51	_	_	GPC 2+ GNB 2+	_	_
52	ENCFE	_	G–B 2+	Vancomycin	Ampicillin/ amoxicillin
53	_	_	_	_	_
54	STRPY	_	_	Penicillin/ ampicillin	_
55	STRPY	_	_	Penicillin/ ampicillin	_
56	_ACIBA _ ESBL KLESP _STRVI	_	_	_colistin _ertapenem _cefotaxime/ ceftriaxone (colistin & ceftriaxone combination for all 3 microorganisms)	_ trimethoprim-sulfamethoxazole; ciprofloxacin; ceftazidime; cefepime; gentamicin piperacillin/ tazobactam; imipenem; meropenem _ trimethoprim-sulfamethoxazole;ampicillin/ amoxicillin; amoxicillin-clavulanic acid;cefazolin; cefuroxime; cefotaxime/ ceftriaxone; ceftazidime; cefepime _

57	STRSP	_	G – B 3+	Ceftriaxone/ cefotaxime	_
58	STRSP (N/S –Spp)	—	_	Ceftriaxone/ cefotaxime	_
59	_SERSP _ENTCL	_	_	_piperacillin/ tazobactam cefepime _piperacillin/ tazobactam; cefepime	_ampicllin/ amoxicillin _ amoxicillin-clavulanic acid;ampicillin/ amoxicillin
60	STRPY	_	_	Penicillin/ ampicillin; clindamycin	_
61	STRAN	_	GPB 1+ GNB 3+	Penicillin	_
62	_ENTCL _ACIBA _SERMA	_	_	_ciprofloxacin; cefepime; ertapenem _ciprofloxacin _ciprofloxacin; cefepime; ertapenem	_ amoxicillin-clavulanic acid;ampicillin/ amoxicillin _piperacillin/ tazobactam; trimethoprim-sulfamethoxazole; meropenem _amoxicillin-clavulanic acid;ampicillin/ amoxicillin
63	ACIBA	_	_	Ciprofloxacin;ceftazidime	Piperacillin/ tazobactam ; meropenem; imipenem
64	STRAN	_	G – B 2+	Penicillin/ ampicillin	_
65	_	_	_	_	_
66	_	_	GPC 3+	_	_
67	_CITFR _ACIBA	_	_	_Ertapenem _Ceftazidime	_amoxicillin-clavulanic acid;ampicillin/ amoxicillin;cefuroxime; ceftazidime _piperacillin/ tazobactam ; meropenem;cefepime; imipenem
68	STAAU	_	_	Cloxacillin; erythromycin/azithromycin	Penicillin/ ampicillin

69	MOGMO	_	GPC 3+ (lost viability)	Ertapenem; cefepime	amoxicillin-clavulanic acid; trimethoprim-sulfamethoxazole; ampicillin/ amoxicillin; cefazolin; gentamicin
70	MOGMO	_	GPC 3+ (lost viability)	piperacillin/ tazobactam	amoxicillin-clavulanic acid;ampicillin/amoxicillin

6.3 Appendix II – Ethics Clearance Certificate

R14/49 Dr Sumaya Ahmed

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M151010

NAME: (Principal Investigator)	Dr Sumaya Ahmed
DEPARTMENT:	Ortorhinolaryngology Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	A 3-Year Retrospective Review of the Microbiology of Deep Neck Infections in Adults at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	30/10/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr P Pillay
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	18/11/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

Date 21/11/15

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

6.4 Appendix III – Supervisor's Acknowledgement of Turn-it-in Report

Supervisor acknowledgement of TURN-IT-IN (Plagiarism) Report

Student name: Sumaya Ahmed

Student Number: 0202217J

Degree: Mmed ORL

Title:

A 7-YEAR RETROSPECTIVE REVIEW OF THE MICROBIOLOGY OF DEEP NECK INFECTIONS IN ADULTS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

TURN-IT-IN (Plagiarism) Report :

ORIGINALITY REPORT:

9% SIMILARITY INDEX

7% INTERNET SOURCES

6% PUBLICATIONS

2% STUDENT PAPERS

Supervisor name: Dr Prebanathan Pillay

Supervisor Signature: P Pillay Date: 18/01/2018