Antibiotic exposure as a risk factor for secondary *Candida* infections in a private hospital intensive care unit

AM Pieterse

orcid.org/0000-0003-4212-9673

Dissertation submitted for the degree *Masters of Pharmacy* in Pharmacy Practice at the North-West University

Supervisor: Dr JM Du Plessis

Co-supervisor: Dr M Julyan

Graduation: March/April 2020

Student number: 20321422

PREFACE & ACKNOWLEDGEMENTS

The words in Philippians 4:6-7 "…do not be anxious about anything, but in every situation, by prayer and petition, with thanksgiving, present your requests to God. [7] And the peace of God, which transcends all understanding, will guard your hearts and your minds in Christ Jesus (Bible,1989)."

This dissertation is in remembrance of my late father, Mr GSM Pieterse, and dedicated to my mother, Ms AE Pieterse.

My sincere appreciation and gratitude towards the following persons who made this possible:

- My promoters: Drs JM du Plessis and M Julyan
- Prof S van Dyk, school director, School of Pharmacy, North-West University and staff, for motivation and making this opportunity possible
- Prof MS Lubbe, the scientific committee of MUSA Medicine Usage in South Africa (MUSA), North-West University, for assistance, motivation and support
- Ms A White for all her support
- Mr J Viljoen for his support and guidance throughout
- The staff of the pharmacy group for everything, big and small, which contributed to the study
- Sr M Bothma for ensuring the quality of all material and for all your assistance.

I would also like to thank the following people who assisted me in various ways, including motivation, friendship and prayers:

My spouse, Mr F Esterhuizen, my relatives Ms M Giliomee, Mr X Giliomee, Ms L Giliomee and Ms A Pieterse; my friends, associates and colleagues, Mr AJ Barnard, Ms D Kuhn, Ms M Basson, and finally, with gratitude, a special thanks to my late father, Mr G Pieterse, who always believed in me – even when I did not believe in myself.

ABSTRACT

Title: Antibiotic exposure as a risk factor for secondary *Candida* infections in a private hospital intensive care unit

Keywords: Antibiotics, antifungal, *Candida,* candidiasis, candidaemia, fungus, intensive care unit, infections, length of stay, mortality, secondary infection

Candida is the most common fungal pathogen and the leading cause of invasive candidiasis in humans. The range of infections caused by *Candida* is vast, and the increased use of potent broad-spectrum antimicrobial agents contributes to the prevalence of worldwide fungal infections.

Antibiotic administration and surgical intervention in patients often result in a profound alteration of protective endogenous flora, promoting overgrowth and translocation of fungi causing secondary invasive infections. Such infections result in a consequent rise in negative patient outcomes and are often associated with high incidences of morbidity, mortality and an overall increase in cost.

This quantitative, cross-sectional cohort study assessed antibiotic exposure as a direct risk for the occurrence of secondary *Candida* infections in intensive care unit (ICU) patients. The study assessed whether the use of, and prolonged exposure to, broad spectrum antibiotics increased the prevalence of secondary *Candida* infections in the ICU of a private hospital located in Klerksdorp, North West Province in South Africa.

The study included a total of 220 patients. Results revealed that 81 patients (85.0%) within the cases group were exposed to multiple antibiotics for a length of stay (LOS) ≥ 7 days. In a stark contrast, the control group displayed a mere 16 patients (15%) with a LOS ≥ 7 days. Prevalence of *Candida* infection was significantly higher in patients exposed to antibiotic treatment for an extended duration ($p < 0.001$).

Antibiotic exposure was identified and evaluated as a direct risk for the occurrence of secondary *Candida* infections in ICU patients.

OPSOMMING

Titel: Antibiotika as ’n risikofaktor vir sekondêre *Candida* infeksies in ’n privaat hospitaal se intensiewe sorgeenheid

Sleutelterme: Antibiotika, antifungus, *Candida,* candidiasis, candidaemia, swam, intensiewesorgeenheid, infeksie, duur van verblyf, mortaliteit, sekondêre infeksie

Candida is die mees algemene patogeniese swam en die hoof oorsaak van indringende candidiasis teenwoordig in die mens. Die omvang van infeksies wat veroorsaak word deur *Candida* is ontsaglik groot en die verhoogde gebruik van sterk, breë spektrum antimikrobiese middels dra by tot die voorkoms van swaminfeksies wêreldwyd.

Die toediening van antibiotika en chirurgiese prosedures veroorsaak dikwels ’n beduidende verandering aan die beskermende endogene flora , dit bevorder die oorgroei en oordrag van swamme wat lei tot sekondêre indringende infeksies. Die gevolge hiervan is ’n aanhoudende verhoging in negatiewe uitkomste vir pasiënte en hou dikwels verband met ’n hoë insidensie van morbiditeit, mortaliteit en ‘n verhoogde koste.

Hierdie kwantitatiewe, deursnit -kohortstudie het blootstelling aan antibiotika as ’n direkte risiko vir die voorkoms van *Candida*-infeksies in die intensiewe sorgeenheid ondersoek. Deur die studie is bepaal of die gebruik van breë spektrum antibiotika en die verlengde blootstelling daaraan die prevalensie van sekondêre *Candida*-infeksies verhoog het in die intensiewe sorgeenheid van ’n privaat hospitaal in Klerksdorp, Noordwes Provinsie, in Suid-Afrika.

Die studie het altesaam 220 pasiënte ingesluit. Resultate het getoon dat 81 pasiënte (85,0%) binne die gevallegroep met 'n LOS ≥ 7 dae, aan veelvuldige antibiotika blootgestel was. In teenstelling het die kontrole groep slegs 16 pasiënte (15%) met `n hospitaal verblyf (LOS) van ≥ 7 dae ingesluit. Die voorkoms van *Candida*-infeksie was aansienlik hoër in pasiënte wat blootgestel was aan antibiotiese behandeling vir 'n lang tydsduur ($p < 0,001$).

Blootstelling aan antibiotika is geïdentifiseer en geëvalueer as ’n direkte risiko vir die voorkoms van sekondêre *Candida*-infeksies in intensiewe sorgeenheid pasiënte.

LIST OF ABBREVIATIONS

30S & 50S	Ribosomal subunits
AIDS	Acquired immune deficiency syndrome
C.	*Candida*
CD_4	Cluster of differentiation 4 extracellular
CI	Confidence intervals
CNS	Central nervous system
CRP	C-reactive protein
CV	Curriculum vitae
DNA	Deoxyribonucleic acid
FISH	Fluorescent in situ hybridisation
GI	Gastrointestinal
GIT	Gastrointestinal tract
HIV	Human immunodeficiency virus
HIV/AIDS	Human immunodeficiency virus/acquired immune deficiency syndrome
HREC	Health Research Ethics Committee
ICU	Intensive care unit
ICUR	Intensive care unit records
IC	Invasive candidiasis
IDSA	Infectious Diseases Society of America
ISO	International Organization for Standardisation
IV	Intravenous

LIST OF ABBREVIATIONS (CONTINUED)

LOS	Length of stay
MALDITOF-MS	Matrix-assisted laser desorption ionisation time of flight mass-spectrometry
MDI	Manufacturer dosage indication
MIC	Minimum inhibitory concentration
MPharm	Master's degree in Pharmacy
MUSA	Medicine Usage in South Africa
NWU	North-West University
PCR	Polymerase chain reaction
QS	Quorum sensing
QSM	Quorum sensing molecules
RNA	Ribonucleic acid
SAP®	Systems, Applications and Products®
SD	Standard deviations
SPSS®	*Statistical Package* for the Social Sciences
spp.	Plural of species
TPN	Total parenteral nutrition

LIST OF DEFINITIONS

Definitions that surface in the integrated literature are defined below in order to enable the reader to develop an understanding of the study terminology.

Antimicrobials	Substances derived from natural, semisynthetic or synthetic basis. These agents can kill (bactericidal) microorganisms or inhibit (bacteriostatic) their growth or replication when optimal concentrations are reached and maintained (Droege *et al.,* 2016:22; Pankey & Sabath*,* 2004:864)*.*
Association	The correlation or a general connection resulting in an interaction or dependence within a framework (Papathomas & Richardson, 2014:48).
Age groups	Age categories and lifecycle groups. Group 1 – children: all children from the age 00 to 14 years. Group 2 – youths: all youths between the ages 15 and 24 years. Group 3 – adults: all adults between the age 25 and 64 years. Group 4 – seniors: all patients aged 65 years and older (Mosby, 2009:1441).
Bactericidal	An antimicrobial agent is considered bactericidal when it is lethal and causes the death of the bacteria (Droege *et al.,* 2016:22; Walsh, 2000:775). Antiseptics, disinfectants and some antibiotics are considered bactericidal substances (Droege *et al.,* 2016:22; Walsh, 2000:775).
Bacteriostatic	These antibiotics mainly impair the growth of the bacteria and inhibit infection (Droege *et al.,* 2016:22; Walsh, 2000:775).
Bioavailability	Bioavailability is a measurement of the rate and extent to which a drug reaches at the site of action. It is expressed in percent, by *F* (Hollenbach, 2008:30; Muilwijk *et al.,* 2014:3294).
Candida	A genus of fungi that are commonly part of the normal flora of the mouth, skin, intestinal tract and are the most common cause of fungal infections worldwide (Marins *et al.,* 2018:187; Manolakaki *et al.,* 2010:367).

LIST OF DEFINITIONS (CONTINUED)

Candidiasis	An infection caused by an opportunistic fungal pathogen, most commonly involving the skin, mucus membranes of the mouth, respiratory tract or vagina. Invasive candidiasis includes candidaemia (Alfouzan *et al.,* 2015:24; Richardson *et al*, 2018:1).
Candidaemia	The presence of any fungus/fungi of the genus *Candida* in the blood or the isolation of *Candida* species (spp.) from at least one blood culture (Colombo *et al.,* 2006:2816; Nieto-Rodriguez *et al.,* 1996:71; Pinhati *et al*., 2016:434).
Colonisation	The invasion of a new habitat by new species. The presence and multiplication of microorganisms without tissue invasion or damage. *Candida* colonisation is the repeated growth of yeasts from at least two different sites (Caggiano *et al.,* 2011:7041). Colonisation is widely recognised as a self-determining risk factor for candidaemia (Caggiano *et al*., 2011:7039).
Concentration dependant	The rate and extent of killing increases as the peak drug concentration increases. A property associated with antibiotics inhibiting protein or DNA synthesis. These antibiotics also exhibit a persistent suppression of bacterial growth after limited exposure to an antibiotic (Pfaller *et al*., 2011:120).
Fever	Normal body temperature is approximately 37ºC, although this varies with the time of day and the method of measurement used. Fever is defined as a higher than normal body temperature. Refractory fever is persistent and resistant to treatment (Kautzky *et al.,* 2015:132; Sardi *et al*., 2013:62).
Fungus	Fungi are a diverse group of eukaryotic, thallus-forming microorganisms (Money, 2015:1). They feed by absorbing organic molecules from their surroundings because they are not capable of photosynthesis. Fungi can reproduce sexually or asexually (McConnaughey, 2014:1). Unicellular fungi reproduce by budding, while multicellular or fungi reproduce by spore formation (Money, 2015:1; Mosby, 2009:1438).

LIST OF DEFINITIONS (CONTINUED)

High-care	A step-down ward within a facility that provides a level of care between general and intensive care unit wards (Prin *et al.,* 2015:1903). The service is designed for relatively intense nursing and continuous monitoring of vital signs and other clinical indicators of a patient's condition and progress (*Miller-Keane* & O'Toole, 2005; Prin *et al.,* 2015:1903).
Intensive care unit	A hospital unit in which there is concentrated specialised equipment and specially trained personnel for the care of seriously ill patients requiring immediate and continuous attention (Prin *et al.,* 2015:1904).
Immunocompromised	A physical state wherein a patient's immune system is compromised (Concia *et al*., 2009:5) and may include but is not limited to, patients with a low cluster of differentiation 4 extracellular (CD_4) count, patients with immune system disorders, patients suffering from immunosuppression due to diabetes, leukaemia, neoplasia, corticosteroid use, antimicrobial therapy, radiation therapy, chemotherapy, malnutrition, malignancy or neutropenia (Fridkin & Jarvis, 1996:501; Pappas *et al.*, 2015:411).
Infection	Invasion by and multiplication of pathogenic microorganisms in a bodily part or tissue, which may produce subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms. Infection is also defined as bacterial or viral colonisation (*Colombo et al., 2006:2816;* Tacconelli *et al.,* 2016:276).
Irrational antibiotic use	The misuse or implementation of antibiotics in an irrational fashion as well as the poor management and regulation of these substances (Bbosa *et al.*, 2014:423). In this study, the misuse or irrational use of medicine is accepted as administration in any manner not aligned with the approved manufacturer's dosage indication or manufacturer's dosage interval.
Length of stay	Length of stay (LOS) is the time measured from the date of admission into the ICU until the date of release from the ICU, transfer to another ward or the date of death (*Miller-Keane & O'Toole,* 2005). Length of stay will be measured in days for the purpose of this study.

LIST OF DEFINITIONS (CONTINUED)

Manufacturer's dosage indication	Manufacturer dosage indication (MDI) for medicine refers to the authorised indication and specific dosage of that drug for treating a particular disease as per development research results. During the process of developing medicine, the manufacturer decides what disease(s) the medicine might treat effectively based on the known effects of the molecule. The manufacturer then conducts studies using people with the disease to determine if the medicine is effective. The studies are designed and conducted under strict guidelines. If the studies show efficacy and no serious side effects, the manufacturer applies for approval of the indication and dosage. When there is enough evidence to approve the medicine for the specific indication, that becomes the approved dosage and indication. The approval means that the manufacturer may include information in their package insert regarding the use of the medicine and the approved dosage for that indication (Thompson & Webb, 2014:866).
Minimum inhibitory concentration	The lowest concentration of a drug that, under established in vitro conditions, inhibits visible growth of a target bacterial population (Mouton, 2012:45).
Mycoses	A fungal infection with environmental and physiological conditions that contribute to the development of fungal diseases (Lass-Flörl, 2009:197).
Neutropenic	An abnormally low level of white blood cells (neutrophil) that is important to fight off infections particularly those caused by bacteria (Tacconelli *et al*., 2016:276).
Nosocomial infections	Infection that can be acquired in a hospital. Infections described as: "Developed in a hospital" or "originating in a hospital" (Akbari & Kjellerup, 2015:458).
Oral thrush	The most common opportunistic infection of the oral mucosa caused by *Candida* spp. characterised by white patches on a red, moist, inflamed surface, occurring anywhere in the mouth, including the tongue, but usually on the inner cheeks, occasionally accompanied by pain and fever (Bugshan, 2017:215, *Carmello et al., 2016:1*).

LIST OF DEFINITIONS (CONTINUED)

Patient outcomes	Patient outcomes are related to mortality, morbidity, length of hospital stay, physiological and clinical outcomes, as well as personal patient-specific outcomes such as quality of life and cost (Concia *et al.,* 2009:11; Curtis, 1998:26).
Prevalence	The number of cases in a population at a given point in time (Mann, 2003:56). Prevalence measures are useful to assess full scope and overall effect (Lund *et al.,* 2015:1601).
Quorum sensing	A communication process involving small secreted molecules. Quorum sensing (QS) is based on two main components, quorum sensing molecules (QSMs) and the increase in cell density of a population. Microorganisms secrete QSMs into the environment and, as the cell density of the population increases, so do the concentration of QSMs. Eventually, the population reaches a tipping point where the QSMs reach a threshold concentration. Once this threshold is met, the population can operate as a 'quorum' and activate signal transduction pathways to activate or repress genes responsible for a variety of biological functions (Antonioli *et al.,* 2018:237; Antunes *et al*., 2010:2282).
Rational (antibiotic use)	The administration and use of antibiotics by a patient that are appropriate for their clinical needs, in doses that meet individual requirements and are limited to an adequate period of time, at the lowest cost to the patient and community (Bbosa *et al.,* 2014:423).
Relationship	A quality that connects two or more things or parts as being or belonging or working together, as being of the same kind or as being logically connected (Glazier *et al.*, 2002:2346; Lund *et al.,* 2015:1601).
SAP®	Systems, Applications and Products®: German enterprise software used to manage business operations and customer service. In this case, the enterprise software used by the data source to manage pharmacy data (Mahadevan *et al*., 2014).
SAP® data	All data captured by the pharmacy on the enterprise software used to manage pharmacy operations and customer service.

LIST OF DEFINITIONS (CONTINUED)

Secondary infection	An infection by a microorganism that follows an initial infection by another kind of organism (*Colombo et al., 2006:2816;* Concia *et al.,* 2009:11).
Virulence	The severity or harmfulness of a disease (Moyes *et al*., 2010:225; Silva *et al*., 2011:289).
Yeasts	Yeasts are single cell eukaryotic fungi that reproduce by various modes of cellular reproduction. They are to convert sugar into alcohol and carbon dioxide (Carmello *et al.,* 2016:1; Kurtzman *et al.*, 2000:29; Walker & White, 2018:4).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

AUTHOR CONTRIBUTIONS

The contribution of each author of the study, titled: Antibiotic exposure as a risk factor for secondary *Candida* infections in a private hospital intensive care unit, is stipulated below:

Author	Role in study
Ms AM Pieterse	Literature review Interpretation of results Writing the final dissertation
Dr JM du Plessis (Supervisor)	Guidance in selection of the research topic Supervision of concept and design of the study dissertation Supervising the writing of the literature review and dissertation Cautious reviewing of the dissertation for intellectual content and final approval
Dr M Julyan (Co-supervisor)	Co-supervision of concept and design of the study dissertation Supervising the writing of the literature review and dissertation Cautious reviewing of the dissertation for intellectual content and final approval
M Cockeran (MSc Statistics School of Mathematical and Statistical Sciences Faculty of Natural and Agricultural Sciences)	Conducted and verified all the results obtained from the statistical analyses

The co-authors confirm their different roles in this study:

I declare that I have approved the above-mentioned dissertation and that my role in this study, as indicated above, is a representation of my actual contribution and I hereby give my consent that it may be published as part of the master's degree in Pharmacy Practice of Ms AM Pieterse.

_____ _____

Dr JM du Plessis Dr M Julyan

_____

M Cockeran

CHAPTER 1: RESEARCH PROTOCOL

1.1 Introduction

Candida is the most common fungal pathogen and the leading cause of invasive candidiasis (IC) in humans (Manolakaki *et al.,* 2010:375; Miller-Keane & O'Toole, 2005). The range of infection caused by *Candida* is considerable, varying from relatively trivial conditions such as superficial candidiasis of the skin and/or mucosal surfaces, to fatal systemic infections such as arthritis, endocarditis and meningitis (McCullough *et al.,* 1996:136; Romani, 2008:529).

Advances in medical technology and the increased use of potent broad-spectrum antimicrobial agents have all contributed to the dramatic increase of fungal infections worldwide (Rinaldi, 1991:493; Vazquez, 2010:80).

Antibiotics remain one of the most frequently prescribed classes of medicines in the intensive care unit (ICU), and are subject to misuse, poor implementation and inadequate management (Ntagiopoulos *et al.,* 2007:363; Pinhati *et al*., 2016:433). Worldwide, the use of antibiotics increased drastically in healthcare in the first decade of the new millennium (Pinhati *et al*., 2016:433).

Application of antibiotics and the trauma associated with surgical intervention cause a profound alteration of the protective endogenous flora, which promotes overgrowth and translocation of fungi resulting in invasive secondary infections (Flanagan & Barnes, 1998:165; Mosby, 2009:1441) associated with a consequent rise in negative patient outcomes and prolonged hospitalisation (Pappas *et al*., 2003:634; Romani, 2008:529).

Although *Candida* infections are more common in those who receive aggressive treatment and undergo invasive procedures, it cannot be regarded as restricted to certain patient groups (Eggimann *et al*., 2003:702; Gong *et al.,* 2016:60), and all patients receiving antimicrobials are a concern (Eggimann *et al*., 2003:689; Gong *et al.,* 2016:60).

The association between the exposure to antibiotics (duration, type and prescribing patterns) and the risk of acquiring secondary *Candida* infections must be investigated to understand the impact of exposure to antibiotics on the occurrence of candidiasis.

1.2 Background to study

Candida species (spp.) are the fourth most common cause of hospital acquired blood stream infections among ICU patients globally (Eggimann *et al.,* 2003:687; Gong *et al.,* 2016:60;

Heimann *et al.,* 2014:331). The incidence of *Candida* infections is usually tenfold higher in the ICU than in any other hospital ward (Bailly *et al.,* 2016:104; Eggimann *et al.,* 2003:687), and accounts for up to 17% of all ICU acquired infections worldwide (Bailly *et al.,* 2016:104).

According to several surveillance studies, the last two decades have seen an increase in the severity and incidence of *Candida*-related infections, with a worrisome trend in related mortality rates (Bailly *et al.,* 2016:104; Kollef & Fraser, 2001:298; Lortholary *et al*., 2014:1303; Pittet *et al.,* 1994*:*752; Vincent *et al*., 2009:2323).

Eight of the 20 *Candida* spp*.* are regarded as clinically important pathogens in human disease: *Candida (C.) albicans, C. tropicalis, C. parapsilosis, C. crusei, C. stellatoidea, C. guilliermondii, C. lusitaniae* and *C. glabrata* (Gong *et al.,* 2016:60; Pappas e*t al.,* 2003:637). These spp. are well known to colonise the skin, the gastrointestinal- and the reproductive tracts (Alfouzan *et al.,* 2015:24), but are not inherently pathogenic for humans (Ho & Cheng, 2010:23; Pittet *et al*., 1994:752).

When patients experience a decrease in both innate and adaptive immune system responses, it results in colonisation. This results in invasion of physiological systems and tissues (Bailly *et al*., 2016:109; Nieto -Rodríguez *et al.,* 1996:71). It furthermore has the potential to develop into life-threatening invasive infections (Karabinis *et al*., 1988:430; Schaal *et al*., 2015:854) such as osteomyelitis, endocarditis or meningitis (Fridkin & Jarvis*,* 1996:502; Pappas *et al.*, 2015:411).

Noni *et al.* (2015:419) observed that there is a clear association between long-term antibiotic treatment and infections caused by *Candida*. Patients exposed to broad-spectrum antibiotics are predisposed to fungal infections, as antibiotics compromise the natural defence mechanisms of the patient (Bassetti *et al.,* 2010:2; Concia *et al.,* 2009:10), breaking down the cutaneous barriers, enabling penetration, which results in colonisation (Bailly *et al*., 2016:109; Blumberg *et al.,* 2001:177; Grewe *et al.,* 1999:412). This study explored whether antibiotic exposure is the direct cause of *Candida* colonisation within the ICU with antibiotic exposure measured by dosage, duration (≥ 7 days or < 7 days), route of administration and quantity.

According to several sources, *Candida* colonisation typically occurs in up to 80% of critically ill patients after seven days in the ICU (Eggimann & Pittet, 2014:1429; Pfaller *et al*., 2011:120-127; Vincent *et al*., 2009:2329), supporting the claim by Caggiano *et al.* (2011:7041) that a prolonged stay in the ICU is one of the most important risk factors for developing IC (Caggiano *et al.,* 2011:7041; Yapar, 2014:102). The relationship between length of stay (LOS) and colonisation is linear (Caggiano *et al.,* 2011:7041; Noni *et al.* 2015:419; Pittet *et al.,* 1994:756), meaning that the longer the patient stays in the ICU the higher the risk of IC becomes (Bessey, 2007:131; Pinhati *et al*., 2016:433). This may be attributed to prolonged exposure to invasive interventions such as

surgery, catheter use, total parenteral nutrition (TPN) and the use of antimicrobial drugs, all predisposing risk factors for colonisation and invasive infections (Yapar, 2014:102).

The purpose of the study is to investigate whether the prevalence of secondary *Candida* infections is directly influenced by antibiotic exposure. It will focus on antibiotic exposure in ICU patients as a risk for developing secondary *Candida* infections in the specified private hospital's ICU, located in the North West Province of South Africa.

1.3 Problem statement

The frequency of secondary C*andida* infections in the ICU setting is on the rise, and candidaemia is an increasing problem in tertiary care hospitals worldwide, especially in patients admitted in the ICU (Lass-Flörl, 2009:197; Pinhati *et al*., 2016:434). Secondary infections cause an increase in discomfort, cost and health-related complications, and drastically decrease quality of life, wellbeing and positive patient outcomes (Concia *et al.,* 2009:11; Pappas *et al*., 2003:634).

From the literature, it is clear that there is an association between antibiotic use and the occurrence of *Candida* infections. Therefore, antibiotic exposure in the ICU was evaluated as a major risk factor for the manifestation of secondary *Candida* infections.

Preventing candidiasis would improve patient safety, result in significant patient outcome improvement (Eggimann *et al*., 2003:702; Romani, 2008:515-529), and can be achieved by implementing effective protocols with the aim to reduce exposure and risk as far as possible in the critically ill patient (Kett *et al*., 2011:668).

1.4 Research aim and objectives

The research aim and objectives supported and highlighted the problem statement.

1.4.1 Research aim

The research aimed to determine the risk of acquiring secondary *Candida* infections as a direct result of antibiotic exposure in the ICU. This was brought about by means of specific literature and empirical research objectives.

1.4.1.1 Specific research objectives

The following specific research objectives guided the process to reach the final aim. Research objectives for the literature review were:

- To define *Candida* infections and review the literature for the epidemiology, risk factors, pathogenesis, clinical presentation, diagnosis and treatment of these infections;

- To review the literature for antibiotic uses, applications and side-effects with a specific focus on risks associated with developing secondary *Candida* infections due to antibiotic exposure;

- To define and clarify the relationship between LOS, antibiotic exposure and secondary *Candida* infections.

1.4.1.2 Empirical investigation objectives

The following specific empirical investigation objectives guided the process to reach the final aim. Research empirical investigation objectives were:

- To determine the prevalence of secondary *Candida* infection in the specific ICU;

- To investigate antibiotic exposure as a risk factor for secondary *Candida* infections.

1.5 Research methodology

Research methods are not merely different ways of achieving the same end. They carry with them different ways of asking questions and enable the researcher to decide on the most suitable design to answer research questions (Babbie, 2010:4).

Retrospective data collection allowed the researcher to use treatment patterns to measure the relationship between antibiotic use and the prevalence of *Candida* infections, in the ICU of a specific private hospital.

1.5.1 Literature review

The literature review involved an intensive study of topic-related literature from reliable sources to collect information regarding the above-mentioned research aims. It aided the researcher in understanding concepts relating to the study and assisted with motivation and clarification of assumptions and results.

Search engines and websites such as EBSCOHost®, Scopus®, Pubmed®, Medscape and Google Scholar™ were consulted to find literature. All scientific information was gathered and filtered for more productive and focused results using keywords and phrases, Boolean and proximity operators and parenthesises. Keywords and phrases such as: ‘Antibiotic*’, *‘Candida’,* ‘candidiasis’, ‘candidaemia’, ‘ICU’, **‘**infection*’, ‘LOS’ ‘mortality’, ‘hospital acquired infection*’,

‘secondary infection*’, ‘antifungal*’, ‘fluconazole’, ‘amphotericin B’ and ‘caspofungin’ formed part of the review process.

1.5.2 Empirical investigation

The study involved the collection of retrospective data to allow exploration of the problem statement within the specific study population. Data were collected for the period 1 January 2013 to 31 December 2016.

1.5.3 Methodological assumptions

The decision to use quantitative methods is satiated with assumptions concerning the nature of knowledge and reality, how one understands knowledge and reality, and the process of acquiring knowledge and knowledge about reality. When one chooses this research approach, one makes certain assumptions concerning knowledge, reality and the researcher’s role.

These assumptions shape the research endeavour, from the methodology employed to the type of questions asked (Babbie, 2010:4). Methodological assumptions give direction to the methods within the study and are the logical application of the scientific methods of the investigation of phenomena (Mouton & Marais, 1996:16).

1.6 Study setting

The study involved the collection of retrospective data related to the treatment of patients in the ICU of a specific private hospital in Klerksdorp, situated in the Matlosana Municipality of the North West Province, South Africa. The specified ICU is established and was not subject to any major or minor renovations during the period 1 January 2013 to 31 December 2016.

At the time of the study, the specified ICU comprised six adult beds, leading medical equipment and technology that were operated by skilled, professional and dedicated employees ensuring that the selected setting was comparable to other settings noted in similar global data surveys (Concia *et al*., 2009:7). During this time, the specific ICU displayed an average occupancy rate of 58% and maintained a staff-to-patient ratio of between 1:1 and 1:2. This ensured consistent optimal care.

It is desirable that a modest descriptive study does not cover many different groups of people (Banerjee & Chaudhury, 2010:62). Homogeneity is ensured when groups with similar ways of life are included within the same cause (Banerjee & Chaudhury, 2010:62), enabling the possibility of defining the population in relation to the specific uniformity (Banerjee & Chaudhury, 2010:63). As a result, the study was restricted to the particular catchment area.

Secondary *Candida* infections have emerged as persistent and dangerous diseases within the ICU globally (Bassetti *et al.,* 2010:1). *Candida*-related infections are far more common in the ICU than in any other ward within the hospital (Bassetti *et al.,* 2010:2). This is largely attributed to the multitude of predisposing risk factors associated with the ICU (Caggiano *et al.,* 2011:7041; Yapar, 2014:102).

The most challenging aspect of a cohort study is the selection of a random sample from a target population in which results can be generalised. To ensure generalisation of results, the population must be clearly defined, and parameters clearly set (Banerjee & Chaudhury, 2010:63). Therefore, to ensure generalisation of results related to this cohort study, clear parameters were set, and the population limited to a specific ICU within a specific geographical area.

Antibiotics are most frequently applied in the ICU (Ntagiopoulos *et al.,* 2007:363; Pinhati *et al*., 2016:433), making it the optimal setting for investigation. The selected setting was suitable for the study as it had implemented an antibiotic stewardship programme and protocols for antibiotic administration resulting in consistent results that aided in meeting the objectives of the cohort study.

Numerous epidemiological data surveys of *Candida* spp. distribution and *Candida* infections in the ICU have been completed in India, Asia, Europe, Canada and the USA (Concia *et al*., 2009:7); however, limited data were available for South Africa and no data had been recorded on this subject for the selected setting, meaning that the results of this study would not only be interesting, but would also be relevant and essential to expand information on local insight and enhance future opportunity for investigations.

Due to the high mortality rate associated with secondary *Candida* infections (Vincent *et al*., 2009:2326), it was decided that selecting the ICU as study setting would add the most value as patients within this ward are at the highest risk for a negative outcome.

1.6.1 Target and study population

The target population included all patients of the specific ICU, admitted and treated during the specified timeframe of the research study. Fungal infections are difficult to treat because antifungal therapy for *Candida* infections is still controversial and based on clinical grounds. As a result, the clinician often assumes that the species isolated from the culture medium is the fungal pathogen (Badiee & Hashemizadeh, 2014:195).

All patients who met the inclusion criteria were used in the study population and an equal number of patients from the same setting, not diagnosed with a secondary *Candida* infection, were included in the study population as a control group.

1.6.1.1 Inclusion criteria

Criteria for inclusion in the study for the cases group:

- All patients admitted to and treated in the study-specific ICU during the study period 1 January 2013 to 31 December 2016 who received antifungal treatment.

Criteria for inclusion in the study for the control group:

- Patients admitted to and treated in the study-specific ICU during the study period 1 January 2013 to 31 December 2016 who did not receive antifungal treatment.

1.6.1.2 Exclusion criteria

Criteria for exclusion from the study for the cases group and the control group:

- Human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) patients, since opportunistic mycoses, such as *Candida*, typically occur in immunocompromised hosts (Eggimann *et al.*, 2011:1), which could have skewed the data.

- Diabetic patients, due to an increased risk of opportunistic pathogens taking advantage of debilitated state of the host's immune system to cause infection (Schaal *et al*., 2015:854; Van Burik & Magee, 2001:744).

- Patients exposed to oncology treatment: In principle, progression is a function of the immune status of the host and the virulence factors produced by the fungus that colonise in the host (Schaal *et al*., 2015:854; Van Burik & Magee, 2001:744) predisposing cancer patients to infection.

- Patients transferred from one facility into this study's setting will be excluded as these patients' treatment history is not accessible by the research team.

1.6.2 Study design

Research methodology refers to the research design chosen to answer research questions (Schmidt & Kohlmann, 2008:165). The design is described in the section below. In the study, a retrospective, quantitative, descriptive, cross-sectional research design was used.

A cross-sectional study, sometimes known as a prevalence study, is a type of observational study that involves the analysis of data collected from a population at one specific point in time (Schmidt & Kohlmann, 2008:165), in this case retrospectively. Retrospective data are collected only once and multiple outcomes can be studied (Mann, 2003:56).

Cross-sectional studies, defined as descriptive in nature, describe risks and occurrence within a population and support inference of cause or effect (Schmidt & Kohlmann, 2008:167). As a result, a cross-sectional descriptive study method is often used to assess the occurrence of illness within a specific population at a specific point in time and to determine the existence and magnitude of independent variables upon a dependent variable at a specific point in time (Schmidt & Kohlmann, 2008:167).

A descriptive study design is created with the intention to provide information regarding prevalence of a variable or its characteristics in a dataset, such as percentages, means and standard deviations. The goal is to present findings on a current situation in a practical reality. During data analysis, the statistical and descriptive relationships are described to answer the research questions (Wood & Ross-Kerr, 2011:115). In this study, the researcher answered the research questions by describing the relationship between antibiotic use and secondary *Candida* infections.

Delivery of results is quick and achieved by determining the odds ratio (Mann, 2003:59; Schmidt & Kohlmann, 2008:167). Subjects are selected without regard to the outcome of interest (Mann, 2003:58).

Quantitative data collection was used as method to collect objective data on the variables of interest out of patient files. The data collected enabled the researcher to associate certain characteristics with adherence or non-adherence.

Subjects were assessed to determine whether they were exposed to the relevant agent (antibiotics) and whether they had the outcome of interest (a C*andida* infection) during the specified study period. The researcher observed, described and documented various aspects of the phenomenon and described what actually existed.

This was done to determine the frequency with which it occurred and to be able to categorise the information (Mann, 2003:59; Schmidt & Kohlmann, 2008:167).

Quantitative research quantifies associations between or among variables. Therefore, the independent or predictor variable (antibiotic administration) and the dependent or outcome variable (occurrence of *Candida* infections) were compared and quantified.

Broadly, quantitative research designs are classified as either non-experimental or experimental. This non-experimental study described, differentiated and examined the associations between the variables and situations. There is no random assignment or manipulation of variables (Mann, 2003:59; Schmidt *&* Kohlmann*,* 2008:167).

Quantitative research utilises methods that objectively collect data and systematically analyse this numerically. Qualitative research is used to describe the relationship between variables and is then used to answer research questions with analysed statistical findings (Mann, 2003:59; Schmidt *&* Kohlmann*,* 2008:167).

The main goal of quantitative research is to produce reliable and valid results that can be generalised to a whole population (Mann, 2003:59; Schmidt *&* Kohlmann*,* 2008:167). In this study, the researcher used retrospective data for numerical analysis to generalise the relationship between administration of antibiotics and prevalence of secondary *Candida* infections.

The researcher did not alter or influence any variables, but collected data as an objective observer; as a result, the study is classified as non-experimental quantitative research (Brink *et al.*, 2012:102). Considering the objectives, the study population and the time available for data collection, the researcher chose a retrospective cohort design.

Because the aim of this research was to determine the risk for occurrence of *Candida* infections due to exposure to antibiotics, this specific study design was used.

1.6.3 Sampling

An all-inclusive sample was used for this study since all patients who meet the inclusion criteria formed part of the study population. For more information regarding the control group, please refer to section 1.6.1.

1.7 Data collection

Study data were obtained from two data sources: the electronic pharmacy computer system data, known as SAP® (Systems, Applications and Products®) data, as well as the ICU patient records (ICURs) only where required.

1.7.1 Data collection sources and tool

SAP® data: Data captured by the pharmacy personnel (pharmacist assistants and pharmacists) on the implemented computer program used in the dispensary. This program is used to record all information regarding stock movement in and out of the pharmacy. The data (retrospective for the

study purpose) were the first source to identify the study population and to collect critical data. Electronic reports were then generated as per the required criteria.

(a) Cases group

(i) The electronic pharmacy records were used to identify all patients who received any medicinal treatment within the specified time period from 1 January 2013 to 31 December 2016 within the ICU.

(ii) It was then filtered further to identify ICU patients who received only antifungal treatment during the specified time.

(b) Control group

(iii) The electronic pharmacy records were used to identify the control group with the same age and gender distribution.

The following variables, for both the cases- and control group, were recorded by the researcher from the SAP® data onto the electronic data collection tool (Annexes A, B & C):

- Treatment regimen. A description of the specific antifungal treatment that patients received in ICU during their stay. This included all antifungal therapy with a detailed description of the dosage of each drug, route of administration, duration and the dosage interval of each treatment.

- Antibiotic treatment. This included all antibiotic therapy with a detailed description of the dosage of each drug, route of administration, duration and the dosage interval of each treatment. This function was used to clarify the treatment regimen of each patient included in the study population to identify all prescribing trends and administration practices of antibiotics in the affected patients.

- Multiple antibiotic treatments used (yes/no): Identification of cases where multiple antibiotic treatments were administered.

Intensive care unit records are hospital documents. Any ICUR data collection was only done in cases where the information was incomplete or in a case where verification of data was required from the SAP® data report.

The ICURs for identified patients were retrieved from the document archive and documents were used to complete data fields and verify information. These documents were collected by the researcher during the data collection session scheduled and only completed after ethics approval

was received. All available data were subject to the exclusion criteria to identify the final study population.

The following variables for the cases- and control groups were recorded by the researcher from SAP® and were referenced to ICU records, where necessary. The following data were captured onto the data collection tool (Annexes A, B & C):

- Demographic information – age and sex excluding any personal information ensuring privacy and personal protection.
- Diagnosis – identification of patients diagnosed with secondary *Candida* infections.
- All information related to the patient's treatment regimen according to the active ingredients (Refer to Annexure G).
- Detailed description of the duration of antibiotic treatment measured between ≥ 7 days or < 7 days.
- Route of administration – intravenous (IV) or oral treatment.
- Length of stay– admission and release dates.
- Dosage – high doses of antibiotic treatment: higher than manufacturer dosage indication (MDI) or low antibiotic doses (lower than MDI).

The study population is the number of people who are theoretically available and included in the research (Wood & Ross-Kerr, 2011:152). It refers to all people in the selected research area who meet the inclusion criteria of the study (Polit & Beck, 2014:178; Wood & Ross-Kerr, 2011:152).

Criteria for exclusion, as described in section 1.6.1.2, were applicable to both the cases group and the control group included in the study, as indicated in exclusion criteria and related variables in Table 1-1.

Table 1-1: Exclusion criteria

Exclusion criteria	Variable(s)
AIDS and HIV patients, since opportunistic mycoses, such as *Candida*, typically occur in immunocompromised hosts (Eggimann *et al.*, 2011:1) and might skew the data.	Diagnosis Existing chronic treatment regimen
Diabetic patients, due to an increased risk of opportunistic pathogens taking advantage of debilitated state of the host's immune system to cause infection (Schaal *et al*., 2015:854; Van Burik & Magee, 2001:744).	Diagnosis Existing chronic treatment regimen
Patients exposed to cancer-related oncology treatment as, in principle, progression is a function of the immune status of the host and the virulence factors produced by the fungus that colonise in the host (Schaal *et al*., 2015:854; Van Burik & Magee, 2001:744), predisposing cancer patients to infection.	Diagnosis Existing treatment regimen Patient history review
Patients transferred from one facility into this study's setting will be excluded as these patients' treatment history is not accessible by the research team.	Patient record review – transfer status check

1.7.2 Validity and reliability of data sources and data collection tool

Electronic data collection decreases the risk or opportunity for erroneous data entry problems, which results in more reliable data collection and results (Worster & Haines, 2004:187-192). In addition, electronic data collection facilitates centralisation and access to data (Gearing *et al*., 2006:126), ultimately resulting in more reliable data and cost-effective collection (Gregory & Radovinsky, 2012:111).

Measures to promote validity commenced at the problem identification stage by clearly defining the research problem, population and variables. Meticulous planning and adherence to detail in every step of the study added to the trustworthiness and validity of the study findings (Burns & Grove, 2009:34).

The use of a retrospective design instead of a prospective design allowed access to a greater study sample in a shorter timeframe, adding to the validity of the study findings. The study design was therefore feasible given realistic time, financial and academic-level constraints (Burns & Grove, 2009:226).

The data reports retrieved from the SAP® system were generated from a validated computer system and all information was checked and signed off by a pharmacist at the point of use. It was therefore accepted as reliable and repeatable. The report was compiled from retrospective data and was prepared by a system administrator reflecting only retrospective data as stored in the server.

As for manual records retrieved from the ICU, data sources were valid and reliable as these were independent private institutions registered and approved by the local governing authority, subject to quality compliance regulations.

The admission and release dates were required, so that LOS could be determined, and treatment details related to specific antifungal treatments had to be recorded in order to determine prevalence in the population.

It was important to collect geographic details from the records in order to effectively stratify results within the population. The data collection tool also required entry of antibiotic-related data. Elaboration on the different application approaches (dose, duration, route, interval etc.) was required, as these influenced the risk and result.

Finally, exposure to different classes of antibiotics was recorded to investigate whether this had any influence on the risk for developing a secondary *Candida* infection. Once the tool was finalised, it was circulated to experts in the field for an accuracy and applicability review.

The statistician and study leaders reviewed the data collection tool to ensure that the correct and relevant data fields were available on the document and that the correct data were collected for the study. Only the researcher collected the data on the data collection sheet. Data collection was done on Microsoft Excel® spreadsheets and a minimum of five random data checks were done by the researcher to verify accuracy of the records and to verify the validity of the data.

1.7.3 Data collection process

The study only commenced once ethical approval was granted, and written goodwill permission was received from the participating hospital. Written goodwill permission was received on 10 January 2017 (Annexure D) on the condition that the study may only continue if ethical approval was granted and therefore data collection was allowed after ethical approval was granted.

Data collection was done electronically on Microsoft Excel® spreadsheets by the researcher outside office hours in the participating hospital pharmacy premises (Klerksdorp) under the supervision of the pharmacy manager. It was done (as described in section 1.7) in the pharmacy manager's office, which is a private lockable room in the relevant private hospital in Klerksdorp. This was to ensure privacy and not to hinder daily operations. Supervision of all activities by the pharmacy manager was a requirement of the participating hospital and a condition for participation in the study. This was due to internal legal privacy and protection requirements.

Data were transferred by the researcher onto the data collection tool by manually populating the required information from the electronic report generated by the SAP® system onto the data collection tool. The pharmacy manager, who is also the appointed responsible pharmacist, did not participate in the collection of data; however, he supervised the process. These steps occurred concurrently.

Electronic data collected were stored on the researcher's personal password- and virus protected laptop. To ensure patient anonymity, unique numbers were allocated to each patient file by the researcher.

Once the data collection tool was manually completed using the electronic pharmacy record, the researcher evaluated whether additional information was required. Any additional data required were collected from the ICUR documents by manually capturing the information onto the electronic data collection tool from the ICUR. Documents related to treatment were available for reference within the pharmacy as all treatment-related retrospective documents are stored in the hospital pharmacy's document store for a minimum of five years as required by local regulation.

The required records were located using the script number reference system implemented in the records store. The relevant document was copied, and the copy was used to manually capture all information on the data collection tool. Once complete, the copy was destroyed via shredding and this was supervised by the pharmacy manager. The researcher used copies for capturing data, ensuring that the original document was not removed from the records store and would at all times be available as required by the hospital staff.

The final part of the data collection process was to supply the electronic data to the statistician for statistical analysis. Data management, during and after the study, will be discussed in section 1.10.8.

Appointments were made with key stakeholders in advance (clinical managers and pharmacy managers) in the specific facility to plan and discuss logistics regarding the data collection process. Upon arrival at the hospital, the data required were accessed from the responsible

pharmacy manager using the hospital computer system SAP®. Using system generated reports, retrospective data were captured onto the data collection tool by the researcher in April 2018.

1.7.4 Recruitment of participants

This was a retrospective quantitative study. No participants were contacted or recruited to partake in the study. All information was collected in the form of retrospective data from the databases, without any patient-researcher contact.

1.8 Study variables

The following variable characteristics were recorded: age, gender, drug therapy, dosage interval, LOS in the ICU and route of administration. As the data were collected retrospectively, the team caring for the patients could not have influenced in the assessment or outcome, as they were unaware of the study objectives.

1.9 Data analysis

Data were processed in collaboration with the statistician, Ms M Cockeran of the North-West University (NWU), Potchefstroom Campus. The statistical program Statistical Package for the Social Sciences (SPSS®) was used to process the data.

The data from the hospital computer system were used to generate reports and the retrospective data were captured onto the data collection tool by the researcher. The data were re-captured in a second back-up worksheet. With the re-capturing of the data in different Microsoft Excel® spreadsheets, possible mistakes could be identified and corrected. Data were analysed by the North-West University's Statistical Consultation Services at the Potchefstroom Campus. The analysed data are presented in Chapter 3.

1.9.1 Statistical analysis

The aim of descriptive statistics is to describe the results of the statistical analysis that is performed. Descriptive statistics break numerous amounts of data into a simple illustrated form (Yokey, 2018:35).

The descriptive part of the analysis is an exploration with the purpose to get a clear overview of the data and the distributions of the variables by diagrams, tables, and basic statistics, such as means and standard deviations (SD) (Hickman & Disler, 2016:132).

Variables for this study were expressed using descriptive statistics such as frequencies (n), percentages (%), means, SD and 95% confidence intervals (CI) (Hickman & Disler, 2016:132).

Cohen's *d*-value was used to determine the practical significance of the results, with $d \geq 0.5$ defined as a medium effect size, $d \geq 0.8$ considered as a large effect size and $d \geq 1.3$ accepted as practically significant, with a very large effect (Cohen, 1969).

The chi-square test was used to determine whether an association exists between two categorical variables (Szucs & Ioannidis, 2017:2). A chi-squared test is any statistical hypothesis test where the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, a chi-squared test often is used as short for Pearson's chi-squared test (Szucs & Ioannidis, 2017:2).

In this study, only descriptive statistics were reported. The use of Cohen's effect size was used to calculate the strength of correlations, and values near 0.1 represent a small strength of correlation, where 0.3 is a noticeable (moderate) strength of correlation, and 0.5 is a practically significant (strong) correlation (Cohen, 1988).

The *p*-value represents the significance of an item. A *p*-value needs to be under 0.05 to be significant. A *p*-value resembles that the difference between variables is not by chance (Hickman & Disler, 2016: 137). Therefore, the *p*-value should be low to indicate a significant difference between the study population and the control group.

The two-sample *t*-test was used to compare the difference between the means of two groups. The two-sample *t-test* for equal means was used to determine if the two-population means are equal. It was applied to compare whether the average difference between two groups was significant or if it is due to random chance. All statistical significance was considered with a two-sided probability of $p < 0.05$, the corresponding confidence level is 95%. The practical significance of results was computed when the *p*-value is statistically significant ($p \leq 0.05$).

If the *p*-value is smaller than 0.05, a statistically significant relationship exists between the two variables; if the p-value is larger than 0.05, there is no statistically significant relationship between the two categorical variables (Cohen, 1969; Szucs & Ioannidis, 2017:4).

Cramer's V is a rescaling of phi so that its maximum possible value is always 1. As the number of rows and columns increases, Cramer's *V* becomes more conservative with respect to phi.

Frequency tables summarise information about one variable, for example demographic data, which describe the characteristics of the research study population (Cohen, 1969). Frequency distribution tables provide a snapshot of the data to allow recognition of patterns. In Chapter 3, frequencies were reported as well as the descriptive statistics related to frequency tables (Cohen, 1988).

Cross-tabulates, commonly referred to as 'crosstabs' is an SPSS® procedure that cross-tabulates two variables, thereby displaying their relationship in tabular form (Cohen, 1969; Yockey, 2018:164). In addition, 'crosstabs' were used to aggregate and jointly display the distribution of variables by tabulating their results one against the other in two-dimensional grids.

'Crosstabs' generated information about bivariate relationships (Cohen, 1969) through the use of SPSS®. It allowed the statistician to calculate and display outputs in simple contingency tables.

1.9.2 Application of statistical tests/measures

The results were evaluated, discussed and tabulated with reference to the aims and objectives set at the beginning of the study. The objective was to relate the results to the literature study. The information gathered in this research addressed the research aims and adheres to the specific objectives.

Recommendations for the implementation of results and further research were formulated. The application of statistical tests and measures is tabulated in Annexure H.

1.10 Ethical considerations

Ethical considerations for the study will be discussed under the below headings:

1.10.1 Application of statistical tests/measures

The research study was subject to approval for submission by the scientific committee of MUSA and thereafter submitted for ethical approval from the Health Research Ethics Committee (HREC) of the NWU. The study only commenced once ethical approval was granted and written goodwill permission was received from the participating hospital.

The institution that participated in this study had to trust that the research team will handle all information and data with respect and absolute confidentiality. All information will be kept confidential and written permission was obtained from the data source (See Annexure E).

A waiver of individual patient informed consent request was made to the HREC for this study since retrospective medical data were be used.

Retrospective analysis of medical records constitutes a notable part of medical research and, in turn, plays a critical role in medical progress (Menon & Cash, 2006:2525). Issues regarding data confidentiality were ethical concerns pertaining to research. For the purpose of the study, the primary ethical issues were overcome by means of:

- Maintaining data confidentiality and
- Ethical and other approval from the relevant authorities.

1.10.2 Anonymity

There was no interaction between the patients and the research team, as the research team collected patient data from the institution retrospectively. There should be no misuse of the information discovered and there should be a certain moral responsibility maintained towards the participants, including the participating institution. This was reflected by implementing absolute anonymity of all participants.

Anonymity of the participating hospital was maintained to prevent stigmatisation. The hospital was not named or referenced during the dissemination of results and the identity of the hospital was kept confidential as per the requirements of the confidentiality agreement. The confidentiality agreement was signed to protect the participating institution and is available – refer to section 1.10.3. The name of the hospital was not published in articles or the dissertation.

Anonymity was achieved by enforcing confidentiality of all data and all information relating to any participants in the study. Anonymity was guaranteed by incorporating a numbering system. This was done by the researcher when populating the data collection tool with data generated by the hospital's electronic system ensuring that the identity of the patient was kept confidential. A random number created to replace the admissions number per patient was unique and ensured that the patient was not identifiable and remained anonymous. There was a duty to protect the rights of people in the study as well as their privacy and sensitivity. No information was published that would be harmful to participants or that could expose any form of identification that would result in prejudice.

1.10.3 Confidentiality

The confidentiality of those involved in the observation had to be maintained, keeping their information and identity secure. It is our ethical responsibility to not harm the humans participating in our study in any way, not only as researchers, but also as pharmacists.

Confidentiality of data was ensured as follows:

- A permission letter was obtained from the data source. The name of the hospital was not published in articles or the dissertation
- Access to the data was subject to signing a confidentiality agreement between the researcher and the data source for this study (Annexure E)
- Data collection took place in the pharmacy manager's office – a private lockable room
- Electronic data were stored on the researcher's password- and virus protected laptop as well as back-up storage on two separate password- and virus protected external hard drives in the possession of the researcher, locked in a fire-proof combination lock safe
- A numbering system was used during data collection ensuring that no individual patient can be identified. This way, the medical record reviews and the analyses of the data were done without revealing the patient identity, ensuring an overall medium risk level for this research study
- Only the research team and the statistician had and will have access to the electronic datasets.

1.10.4 Justification of research study

It was necessary to investigate the effect of antibiotic use on the prevalence of secondary *Candida* infection to be able to determine whether additional precautions regarding C*andida* infection prevention should be implemented when considering antibiotic treatment in ICU patients.

This research endeavoured to address gaps in the existing knowledge base. The study contributed to a new perspective about the risks of antibiotic use and the occurrence of *Candida* infections.

1.10.5 Respect for research participants

The study population was the vulnerable party in the study and needed to be protected from any harm and exposure. Respect for the study population as individuals with the right to privacy and confidentiality was of great importance. The study team endeavoured to respect the rights of the selected patients by handling all data as private and confidential as described in section 1.10.3. Furthermore, all records had the same chance of being included into the study population.

1.10.5.1 Process of obtaining informed consent

A waiver of informed consent was approved by the HREC for this study since retrospective medical data were used.

1.10.5.2 Benefit-risk ratio analysis

In order to comply with the necessary ethical requirements, it had to be demonstrated that all the benefits and risks that participants may experience in the study were considered. The benefits of participating in a study must always outweigh the risks and risks should always be minimised or avoided (Mayan, 2001:31).

1.10.5.3 Anticipated benefits

The anticipated benefits of the study will be discussed under the direct and indirect benefits:

1.10.5.4 Direct benefits

There were no direct benefits for participants as this was a retrospective study and there was no contact between the researchers and participants.

1.10.5.5 Indirect benefits

The indirect benefits of this study include the following:

- Contribution to science and participants in the understanding of antibiotic exposure as a risk factor for the development of secondary *Candida* infection.
- The possible publication of the results in medical journals and the presentation of the results at conferences can contribute to healthcare practitioners' knowledge on the factors of antibiotic prescribing that can result in secondary *Candida* infection.

1.10.6 Anticipated risks and precautions

There were no direct risks for participants as appropriate measures were taken to ensure protection of information and confidentiality. The benefits for the outcomes of this study outweighed the risks. The anticipated risks and the precautions taken are summarised in Table 1-2.

Table 1-2: Anticipated risks and planned precautions

Anticipated Risks	Precaution
Possible negative exposure for the health institution that acted as data source.	A confidentiality agreement was signed between the researcher, pharmacy and the hospital (the hospital provided access to data and owns the data). Safe storage of all electronic data as committed by the research team. The study focused on quantitative data collected in previous years and therefore the study did not undermine the pharmacy and hospital's current healthcare services.
Possible loss of anonymity due to exposure of personal information accessed by the researcher during data collection.	Access to the data was subject to the requirements of the confidentiality agreement.
Loss of time for the researcher.	The researcher developed timelines and collection dates to limit unnecessary time loss.

This study is a medium-risk study since retrospective data were used.

1.10.7 Reimbursement of study participants

The study was based on retrospective data; no participant was contacted or reimbursed for participation. The pharmacy manager supervised the data collection process due to the requirement of the participating hospital and made his office available after office hours as per the conditions stipulated by the participating hospital. The pharmacy manager received standard compensation for his after-hour participation.

1.10.8 Data management

Data management will be discussed in detail under the following headings.

1.10.8.1 Data management during the data collection phase

Only electronic data were collected; no hard copies were collected during the study. During the data collection phase, the electronic data were stored on the researcher's password- and virus protected laptop as well as back-up storage on two separate password- and virus protected

external hard drives in the possession of the researcher. The password protected electronic dataset was made available to the research team and the statistician.

1.10.8.2 Data management after the completion of the study

After completion of the study, all the data were supplied to MUSA and stored on a designated hard drive at the NWU for a minimum of seven years. Data will only be deleted after the required retention period and this will be done under the direct supervision of Ms A Bekker, the appointed research assistant and permanent employee of the research entity, MUSA. The data on the researcher's laptop and two external hard drives will be deleted under the direct supervision of the research assistant of MUSA.

All records will be treated as confidential and will be handled in strict accordance with the rules and regulations of the HREC of the NWU as well as the Scientific Committee of MUSA.

1.10.9 Dissemination of research results

Results were published in a formal dissertation to the NWU after approval, and printing was completed in order to complete a Master of Pharmacy in Pharmacy Practice.

1.10.10 Limitations of the study

The limitations to the study that affected the outcome of the results or areas that could be improved in future studies are discussed in section 4.4.

1.10.11 Role and experience of the members in the research team

The researcher (Ms AM Pieterse) is a pharmacist who has experience in the hospital sector of South Africa as dispensing pharmacist. The researcher was also responsible for data collection, -management and -analysis.

The roles and experience of members of the research team are summarised in Table 1-3.

Table 1-3: Roles and experience

Name	Role in the study	Expertise and experience
Ms A Pieterse Master's in Pharmacy (MPharm) candidate	Literature study research, data collection, interpretation of results, writing and compiling this dissertation.	The researcher was an active member of the antibiotic stewardship programme implemented at her previous place of employment and contributed to the responsible use of medicine through clinical dispensing and counselling. The researcher completed a research methodology course and ethics training as part of the Master's in Pharmacy (MPharm) programme. The researcher is currently employed as a quality and compliance specialist with a global pharmaceutical company and frequently leads validation and qualification of systems. The researcher has completed the International Organization for Standardisation (ISO) training and is qualified as a quality management system and validation specialist. Please refer to the narrative curriculum vitae (CV) of the researcher.
Dr JM Du Plessis (Supervisor)	Supervising the MPharm student and active monitoring of the research project.	Please refer to the narrative CV of the researcher.
Dr M Julyan (Co-supervisor)	Supervising the MPharm student.	Please refer to the narrative CV of the researcher.

1.10.12 Monitoring of the research project

A half-yearly monitoring report was submitted to the HREC of the Faculty of Health Sciences of the NWU. Amendments with all supporting documentation and required information were submitted to the above-mentioned committee.

The research project implementation was monitored by the study timeline and project plan, which clearly defined the timelines for each research activity and stated who was responsible for completing the activities. The project plan was an official dissertation work schedule governed by an evaluation cycle. Each activity was evaluated for compliance and conformity and this involved reflecting, learning and adjusting before moving to the next action ensuring a continuous motion of constant reflection resulting in effective monitoring.

1.10.13 Conflict of interest

There was no conflict of interest. The research team was independent and objective with regard to results and implications of this study. There was no monetary or any other form of compensation given as incentive to researchers to be part of the research team. There was no monetary or personal gain for any member of the research team as an incentive to participation in the study. The pharmacy and hospital management received no monetary or any other form of compensation for providing the study setting and data source. The researcher was not an employee of the pharmacy or hospital where the study took place.

1.11 Study layout

The study layout is the framework that was created to find answers to research questions. The study is designed as follows:

- **Chapter 1**: Research Protocol
- **Chapter 2**: Literature review
- **Chapter 3**: Data analysis and results
- **Chapter 4**: Conclusions, recommendations and limitations.

1.12 Summary

Chapter 1 introduced secondary *Candida* infections in the ICU as a worldwide concern, which contributes to morbidity and mortality. The proposed method to identify the impact of antibiotic use on the prevalence of secondary *Candida* infections will use a structured design to answer the

research questions. The chapter also included a full description of processes to ensure that high ethical standards are maintained.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

"Whereas in the past, opportunistic mycoses, such as Candida, typically occurred in immunocompromised hosts, these complications are increasingly observed in non-immunocompromised critically ill patients…It is very difficult to predict the incidence of invasive candidiasis and early diagnosis remains a major challenge…

Accordingly, clinicians should continue to combine risk factors and the dynamic of colonisation to try to implement early treatment or prevention."

- Philippe Eggimann, 2011 -

Candidiasis, defined as an infection by yeasts of the genus *Candida*, is the leading cause of fungal infections in humans (Manolakaki *et al*., 2010:375) and has become an important form of invasive mycoses in the ICU (Eggimann *et al*., 2011:1; Lass-Flörl, 2009:197; Pappas *et al*., 2003:634).

Candida spp. are known to reside on various surfaces in the body (Alfouzan *et al*., 2015:24; Pappas, 2006:485). It is present in all humans and has the potential to infect any patient. Therefore, it is very important to establish the risk factors for candidaemia, which occurs when *Candida* enters the bloodstream of a patient (Eggimann, *et al.*, 2003:685; Gong *et al*., 2016:60) in order to minimise the risk of exposure and prevalence. Candidaemia is usually associated with a consequent rise in related mortality rates and a prolonged LOS, especially in the ICU (Pappas *et al*., 2003:634; Romani, 2008:529).

Fungal colonisation of the skin and mucosa is a prerequisite for fungal dissemination and proliferation in tissues (Bailly *et al*., 2016:109; Blumberg *et al*., 2001:177; Grewe *et al*., 1999:412), and so ultimately the prerequisite for the development of candidaemia (Caggiano *et al*., 2011:7041; Yapar, 2014:102). This clearly indicates that exposure to risks resulting in colonisation will enhance the prevalence of systemic infection (Caggiano *et al*., 2011:7041; Yapar, 2014:102).

One of the major risk factors for colonisation is antibiotic use (Flanagan & Barnes, 1998:165; Mosby, 2009:1441; Yapar, 2014:102). Antibiotics and the trauma associated with surgical intervention are known to cause a profound alteration of the endogenous flora in the body, which promotes overgrowth and translocation resulting in colonisation and infection (Eggimann *et al*., 2003:689; Flanagan & Barnes, 1998:165; Gong *et al*., 2016:60). This chapter will focus on a clinical review of *Candida*, antibiotics and the relationship between these entities to answer the research questions.

2.2 Background

In 2017, based on population and disease demographics, it was estimated that over 1 billion people were afflicted with fungal infections worldwide (Cornely *et al*., 2017:420). Bassetti *et al*. (2010:2) established that *Candida* infections are one of the most frequent and most serious infections in patients admitted to the ICU with global models estimating that IC affects more than 700 000 people per year (Pfaller *et al*., 2011:123).

When presenting as invasive candidaemia, the infection results in high incidences of mortality (Bailly *et al*., 2016:104; Romani, 2008:529; Vincent *et al*., 2009:2327) with rates ranging between 29% and 76% and an attributable mortality rate as high as 49% in patients admitted to the ICU (Heimann *et al*., 2014:331; Vincent *et al*., 2009:2327).

The incidence of *Candida* infection increases along with the augmented use of invasive measures (Gong *et al*., 2016:60) such as IV catheters, administration of broad-spectrum antibiotics and the use of TPN (Eggimann *et al*., 2003:702; Gong *et al*., 2016:60).

Invasive measures cause translocation of fungal organisms (Caggiano *et al*., 2011:7041; Yapar, 2014:102) through the suppression of normal bacterial flora allowing indigenous fungi to proliferate and colonise (Bailly *et al*., 2016:104; Vincent *et al*., 2009:2323).

Antibiotics promote fungal overgrowth by suppressing the growth of the normal aerobic flora (Flanagan & Barnes, 1998:165; Mosby, 2009:1441) and enabling the high local concentrations of *Candida* spp. to translocate across mucosa to enter the bloodstream (Karabinis *et al*., 1988:430; Schaal *et al*., 2015:854). The risk increases exponentially with each class of antibiotic added in treatment (Bassetti *et al*., 2010:2; Concia *et al*., 2009:10).

Existing literature was used to investigate the effect of antibiotic administration on the prevalence of *Candida* infection and to review the microbiology, epidemiology, signs, symptoms and treatments associated with *Candida* infections.

It furthermore focuses on antibiotic medicines by researching the classes, uses, dosages, duration and side effects of these medicines with the goal of understanding the impact that antibiotics have on the prevalence of secondary *Candida* infections.

2.3 Candida

Two centuries of medical history were recorded before the etiological agent of oral thrush (a *Candida* infection of the oral mucous membrane) was correctly identified as the fungal pathogen *Candida* (Carmello *et al.,* 2016:1*;* McCullough *et al.,* 1996:136; Romani, 2008:529).

A recent review of the early taxonomy of *Candida* reported that it was not until the mid-19th century that the clinical nature of oral candidiasis was defined and correctly described (McCullough *et al.,* 1996:136; Romani, 2008:529).

Candida was first described in 1839 and the name was derived from the Latin word *candidus*: meaning 'gleaming white', describing the white exudate present on the infected mucosal surfaces of affected patients (Gow & Yadav, 2017:1146).

The genus *Candida* belongs to the class *Deuteromycetes* and has been described as a 'taxonomic pit' into which yeasts without a known sexual stage or other remarkable phenotypic character have been thrown (McCullough *et al.,* 1996:136; Romani, 2008:529). Its members are biologically diverse and include yeasts with ascomycetes and basidiomycetes affinities (McCullough *et al.,* 1996:136; Romani, 2008:529).

Within the genus, species are characterised primarily by colonial morphology, carbon utilisation and fermentation (Gallis *et al*., 1990:308). The genus *Candida* includes characteristically white, imperfect yeasts capable of forming pseudo-hyphae, the result of incomplete budding where the cells elongate, but remain attached after division (Saville *et al*., 2003:1060).

In healthy individuals, *Candida* spp. assists the body and aids in the digestion of food and absorption of nutrients (Kullberg & Arendrup, 2016:795). *Candida* spp. ordinarily exists in balance with trillions of bacteria that inhabit a healthy digestive tract (Gow & Yadav, 2017:1146). *Candida* infections (candidiasis) are very infrequent in healthy individuals (Moyes *et al*., 2010:224).

Candida is universally present on mucosal surfaces and healthy skin of adult humans in small quantities (Gow & Yadav, 2017:1146). Between 30 and 70% of healthy individuals carry at least one *Candida* species commensally (Kullberg & Arendrup, 2016:795), although it has been shown that individuals may harbour more than one strain of *Candida* at the same time (McCullough *et al.,* 1996:136; Romani, 2008:529).

Intra-oral commensal existence of *Candida* occurs in at least 50% of the population and the gastrointestinal tract (GIT) is another major habitat of the commensal *Candida* spp. (McCullough *et al.,* 1996:136; Romani, 2008:529). Population level analysis of GIT microbiota reveals gender-

related differences, with female subjects showing a higher number of fungal isolates and fungal species compared to male subjects (Strati *et al*., 2016:1227).

In this commensal environment, fungi are constantly challenged by the host and other microbial habitants, resulting in evolved commensal factors, which can turn into virulence attributes once the conditions favour infection (Whittington *et al.*, 2014:3).

There are several virulence factors for *Candida* that promote successful colonisation or invasion of host tissues and many have been described (McCullough *et al.,* 1996:136; Romani, 2008:529). This being noted, not all hosts are equally susceptible to microbial infections and even closely related fungal spp. can have very different ecologies and relationships with their hosts (Whittington *et al.*, 2014:4).

Although virulence is due to microbial attributes, these attributes are only expressed in a susceptible host (Casadevall & Pirofski, 2007:24) and, ultimately, susceptibility is defined by the host spp. and is influenced by the immune status of a host (Whittington *et al.*, 2014:4).

When the skin or mucosal barriers are damaged, it becomes amenable for colonisation and rapid growth (Caggiano *et al*., 2011:7039; Wey *et al.,* 1988:2649). In the event that the immune systems are weakened, for example when the competing flora are eliminated after antibiotic treatment, *Candida* colonises and invades host tissues (Schaal *et al*., 2015:854; Van Burik & Magee, 2001:744).

Candida is very well adapted for life as commensal spp. (Moyes et al., 2010:224). The evolutionary forces that have sculpted the genome of this fungus have resulted in a gene set that enables the fungus to be retained in a commensal state on the mucosa when the immune system is vigorous and intact (Whittington et al., 2014:14), and simultaneously to flourish when the host immune surveillance is compromised (Alfouzan et al., 2015:24; Pappas, 2006:485). This makes *Candida* an extremely dangerous pathogen (Mosby, 2009:1438; Moyes et al., 2010:225; Silva et al., 2011:289).

2.4 Epidemiology

Over the past 10 years, some studies have reported a shift in the aetiology of candidaemia, while C. *albicans* is still considered the most common species causing candidaemia. Increasing rates of candidaemia caused by *C. tropicalis, C. parapsilosis, C. glabrata* and *C. krusei* have been reported (Bassetti *et al*., 2010:4, Caggiano *et al.,* 2011:7041; Yapar, 2014:102).

Several epidemiological studies have revealed emerging spp. that may vary geographically in frequency of isolation (Eggimann & Pittet, 2014:1429; Pfaller *et al*., 2011:120-127; Vincent *et al*.,

2009:2329). In a global study completed by Kett *et al,* which included 76 countries, the prevalence per species was reported as follows: C. *albicans* at 65.1%, C. *glabrata* at 13.1%, C. *tropicalis* at 6.2%, C. *krusei* at 4%, C. *guilliermondii at* 0% as well as C. *parapsilosis* at 0.6% (Kett *et al*., 2011:665).

Candida albicans remains the predominant cause of invasive fungal infections worldwide (Horn *et al*., 2009:1703), although the incidence of infections due to non-*albicans* spp. is increasing steadily (Sardi *et al*., 2013:11).

Species such as *C. parapsilosis* have emerged as significant nosocomial pathogens with clinical manifestations that include endocarditis, septic arthritis and peritonitis (Fridkin & Jarvis, 1996:502). *Candida tropicalis* has been associated with candidaemia in cancer, especially in patients with leukaemia or neutropenia (Colombo *et al*., 2006:2823), and C. *glabrata* has been reportedly related to candidaemia due to the use of fluconazole (Colombo *et al*., 2006:2823).

There is mounting evidence suggesting that candidaemia has become a major problem in ICUs worldwide (Colombo *et al*., 2006:2816; Nieto-Rodríguez *et al*., 1996:71; Pinhati *et al*., 2016:434). This has been particularly evident among patients hospitalised for long periods of time (Colombo *et al*., 2006:2816).

Candida spp. is currently the third most frequent causative agent of late-onset sepsis in the ICU (Droege *et al*., 2016:22), and the most common causative pathogen of invasive fungal infections worldwide (Manolakaki *et al*., 2010:367). In the ICU, candidaemia ranges from five to ten cases per 1 000 admissions or three to 15 incidences per 10 000 patient days, usually tenfold higher than in general hospital wards (Bailly *et al*., 2016:104; Eggimann *et al*., 2011:7).

The rise of incidence in infection represents a serious public health challenge with increasing medical and economic importance (Pfaller & Diekema, 2007:163), exasperated by the fact that candidaemia is difficult to diagnose and hard to treat (Colombo *et al*., 2006:2816).

2.5 Pathogenesis & Microbiology

Although *Candida* normally colonises mucosal surfaces in an asymptomatic manner, it can become a significant cause of disabling and lethal infection (Heimann *et al*., 2014:331; Vincent *et al*., 2009:2323). *Candida* spp. is especially virulent due to its exceptional adaptability to different host niches (Sardi *et al*., 2013:10).

Candida spp. is able to transition between yeast, pseudo-hyphal- and hyphal phenotypes (Saville *et al*., 2003:1060). This change is spontaneous and reversible (Sardi *et al*., 2013:10) and makes the spp. highly adaptable in changing environments (Shapiro *et al*., 2011:267). This feature

enables *Candida* to grow in two different ways: reproduction by budding as well as in hyphae form (Shareck & Belhumeur, 2011:1012).

Candida can create a biofilm as it becomes multicellular. The biofilm is made primarily of cellulose, but also contains polynucleotides, polypeptides and fibrinogen (Rajendran *et al*., 2010:229). This allows *Candida* to switch between different morphologies, largely dependent on environmental cues (Shareck & Belhumeur, 2011:1012).

These cues include: temperature, nitrogen levels, amino acid availability, pH, nutrient limitations, serum, quorum sensing and solid matrixes (Kim & Sudberry, 2011:172). Each environmental cue has an associated membrane detector or directly interacts with a genetic factor of the respective signalling system (Kim & Sudberry, 2011:172).

Each morphological state is associated with specific roles in *Candida* pathogenesis (Kim & Sudberry, 2011:173). To be effectively virulent, it is essential for *Candida* to interconvert between morphologies (Kim & Sudberry, 2011:173).

Candida spp. is an eukaryotic opportunistic pathogen (Kim & Sudberry, 2011:171). The yeast form of *Candida* is Gram-positive, and a transcription repressor is needed to maintain this state (Grubb *et al*., 2008:4370). The yeast morphology is necessary for initial attachment, colonisation and dissemination of the organism at the site of infection (Saville *et al*., 2003:1060).

This eukaryotic organism is composed almost exclusively of molecules that are absent from the human body and are therefore a key target for immune recognition (Romani, 2008:529). The cell wall is essential for viability and one sixth of the total genome is invested in its biosynthesis and maintenance (Kim & Sudberry, 2011:173).

The cell wall is highly dynamic and the changing nature of the cell surface presents challenges for the detection and surveillance of the fungus (Sardi *et al*., 2013:10). This is important in terms of commensalism and pathogenicity (Kim & Sudberry, 2011:173).

Cell wall proteins play essential roles in modulating the host immune response (Bailly *et al*., 2016:859; Blumberg *et al.,* 2001:177). The wall exists in two readily distinguished layers: an outer layer that is enriched with glycosylated glycoproteins (Shareck & Belhumeur, 2011:1012), and the inner skeletal cell wall that is composed predominantly of robust polysaccharides (Shareck & Belhumeur, 2011:1012).

The hyphal form has the crucial responsibility of host invasion (Shareck & Belhumeur, 2011:1012). Invasion requires the hyphal form to breach epithelial cells and their associated barriers to enter tissue or the bloodstream (Grubb *et al*., 2008:4370).

Candida is commensal in humans and therefore the host immune system has adapted to recognise when *Candida* shifts to a pathogen form (Moyes *et al*., 2010:224). The yeast form adheres to host cells and is tolerated, but when the organism switches to hyphal morphology, this change alerts the host and elicits an immune response (Moyes *et al*., 2010:225).

2.5.1 Infections

Infection is defined as the invasion of an organism's body tissues by disease-causing agents, their multiplication and the reaction of host tissues to these organisms and the toxins they produce (Colombo *et al*., 2006:2816; Tacconelli *et al*., 2016:276).

Candidiasis is caused by colonisation that results in translocation and infection (Eggimann *et al*., 2003:689; Flanagan & Barnes, 1998:165; Gong *et al*., 2016:60). *Candida* infections show a wide spectrum of clinical presentations and can be classified as superficial or severe and invasive (Bassetti *et al*., 2010:2; Eggimann *et al*., 2003:687).

Invasion results in superficial infection, known as candidiasis, and often presents as thrush of mucosal tissues (Carmello *et al.,* 2016:1). In the event that the organism then gains further access and enters the blood stream (a condition known as candidaemia), the infection progressively spreads from one organ in the body to another, affecting a variety of systems (Tacconelli *et al*., 2016:276). This aggressive progression can result in serious systemic infection and even death (Colombo *et al.,* 2006:2816; Groll *et al*., 1998:343; Nieto-Rodriguez *et al.,* 1996:71; Pinhati *et al*., 2016:434).

When infections spread, they are classified as invasive infections. Serious IC is variously described as deep-seated, invasive, systemic, disseminated, haematogenous and haematogenous-disseminated, and mortality due to candidaemia is extremely high, with some centres reporting mortality in excess of 40% (Gow & Yadav, 2017:1146).

Invasive candidiasis describes two close but distinct entities: candidaemia and systemic candidiasis (Colombo *et al*., 2006:2816; Nieto-Rodríguez *et al*., 1996:71). Invasive candidiasis can be defined as a deep-seated disease that frequently involves multi-organ infections although blood cultures may be negative (Tacconelli *et al*., 2016:276). A definite fungal infection is present when tissue invasion is proven via biopsy or culture of samples obtained from a sterile site (Hollenbach, 2008:26; Tacconelli *et al*., 2016:276).

Candidaemia, in turn, refers specifically to the isolation of *Candida* spp. in blood (Pinhati *et al*., 2016:434). If the patient presents with temporary related signs of infection, candidaemia is proven (Colombo *et al*., 2006:2816; Nieto-Rodríguez *et al*., 1996:71; Pinhati *et al*., 2016:434). It is also

considered candidaemia when isolation of *Candida* spp. is established within the pleural-, pericardial-, peritoneal- or cerebrospinal fluid (Kett *et al*., 2011:665).

Virulence factors are defined as the severity or harmfulness of a disease (Moyes *et al*., 2010:225; Silva *et al*., 2011:289). *Candida* has numerous virulence factors to enhance survival in the host for both localised and systemic infections (Moyes *et al*., 2010:225; Silva *et al*., 2011:289).

Virulence factors, such as the ability to evade host defences, adherence to host tissue and on medical devices, the ability to switch between the yeast and the hyphae mode, as well as the production of tissue-damaging hydrolytic enzymes make *Candida* an extremely dangerous pathogen (Mosby, 2009:1438; Moyes *et al*., 2010:225; Silva *et al*., 2011:289).

These virulence factors enable the fungus to invade into deeper tissue or gain access to blood vessels via damaged barriers. Fungal cells then enter the blood stream and escape through the endothelial layers to colonise the major organs, causing life-threatening systemic infections (Whittington *et al.*, 2014:3).

2.5.2 Classification of infections

Fungal pathogens can be classified into two classes of pathogens: primary- and secondary opportunistic pathogens (Alfouzan *et al*., 2015:24; Pappas, 2006:485). Primary pathogens have an environmental reservoir and infect individuals who have been exposed or who are immunologically naïve to fungi (Van Burik & Magee, 2001:744). Secondary opportunistic pathogens take advantage of debilitated or immunocompromised hosts to cause a secondary infection (Schaal *et al*., 2015:854; Van Burik & Magee, 2001:744).

A primary infection usually involves an initial cause (bacterial, viral, microbiological exposure) resulting in an infection that leads to a physiological impact of the host (Colombo *et al*., 2006:2916). The primary infection is the original cause of infection or admittance of the patient to the hospital (Prin *et al*., 2015:1903).

A secondary infection is an infection that occurs during or after treatment of another already existing infection. It may result from the treatment itself or from alterations in the immune system. A secondary infection is an infection that occurs during or after treatment of another pre-existing infection (Prin *et al*., 2015:1903).

Primary infections are caused due to overgrowth of these organisms resulting in symptoms. These symptoms may vary depending on the area of the body that is infected. However, when the integrity of skin or the GIT mucosa is compromised, it may cause penetration by the yeast, leading to a secondary systemic infection (Bassetti *et al*., 2010:2).

Infiltration occurs when hyphae invade through epithelial cells and deep into epithelial layers. Secretion of hydrolases, hyphae associated damaging factors and hyphal extension (Mosby, 2009:1438; Moyes *et al*., 2010:225) then contribute to damage of host tissue, exposing the patient to secondary infection (Colombo *et al*., 2006:2916).

Additional tissue damage then occurs because of an overreaction of the immune system and a massive infiltration of neutrophils, which releases toxic agents and results in additional damage to epithelial cells (Colombo *et al*., 2006:2916).

Candida spp. can be the cause of both primary or secondary infections, depending on the nature of the underlying host defect (Mosby, 2009:1438; Moyes *et al*., 2010:225). This is a key characteristic of pathogenesis and disease (Colombo *et al*., 2006:2916).

2.5.3 *Candida* in the intensive care unit

The ICU was first introduced in the 1950s (Wenham & Pittard, 2009:178) and this specialty care medicine unit was developed because of the poliomyelitis epidemic, when widespread mechanical ventilation was required (Wenham & Pittard, 2009:178).

The ICU is a hospital unit in which there is concentrated specialised equipment and specially trained personnel for the care of seriously ill patients requiring immediate and continuous attention (Prin *et al*., 2015:1904).

The specially trained ICU staff are responsible for the care of patients with severe and critical illnesses and injuries that require intensive monitoring and support from specialist equipment and medications in order to ensure survival and recuperation (Prin *et al*., 2015:1904). They are highly qualified professionals who specialise in caring for critically ill patients. The higher staff-to-patient ratio and access to advanced medical resources and equipment, which are not routinely available elsewhere, distinguish this ward from other wards (Miller-Keane & O'Toole, 2005:1960).

Since the establishment of the ICU, the technology available to support the critically ill patient has become more sophisticated and complex (Wenham & Pittard, 2009:178). These advances in medical science allow patients with severe and complicated diseases to survive, but create a population of subjects vulnerable to a range of opportunistic infections (Bassetti *et al.,* 2010:2). Where, as in the past, opportunistic mycoses, such as *Candida,* typically occurred in immunocompromised hosts, it is increasingly observed in non-immunocompromised ICU patients (Eggimann & Pittet, 2014:1429; Pfaller *et al*., 2011:120-127; Vincent *et al*., 2009:2329).

The emergence of candidaemia among hospitalised patients is associated with the use of broad-spectrum antibiotics, as well as the increasing number of patients with long ICU stays (Bassetti

et al., 2010:2). Candidaemia most often occurs after a long ICU stay (Leroy *et al*., 2009:1616), which makes patients vulnerable to opportunistic infections (Leroy *et al*., 2009:1616). Patients who remain in the ICU for extended periods of time have a much higher prevalence of multi-organ failure, higher mortality rates and high resource consumption (Yapar, 2014:101).

The incidence of candidaemia is highest in the ICU (Leroy *et al*., 2009:1616). *Candida* infections may represent up to 15% of nosocomial infections and the crude mortality rate has been found as high as 60%, varying according to the study design and the population, with the estimated attributable mortality as high as 47% (Bassetti *et al.,* 2010:2). More than two thirds of patients with systemic candidiasis in ICU present with candidaemia (Leroy *et al*., 2009:1616).

Candidaemia generally occurs in patients who are debilitated and the risk factors for debilitation are common in the ICU (Bassetti *et al.,* 2010:2). The predominant source of IC infections in the ICU is endogenous (Bassetti *et al.,* 2010:2). Numerous conditions frequent in ICU patients may damage the integrity of skin or the gastrointestinal mucosa leading to systemic infection (Leroy *et al*., 2009:1616).

A wide variety of environmental factors affect patients in the ICU and they all interact with each other having significant effects on the health, well-being and outcome of patients (Wenham & Pittard, 2009:182). The ICU is an inherently stressful setting (Wenham & Pittard, 2009:178). It is a potentially hostile environment to the vulnerable critically ill patient. In addition to the physical stress of illness, pain, sedation, interventions and mechanical ventilation, there are also psychological and psychosocial stressors that predispose these patients to secondary infections (Wenham & Pittard, 2009:178).

Although IC infections can affect any hospitalised patient, they are more common and have unique attributes in the ICU (Leroy *et al*., 2009:1616). As a result, many studies have been conducted on ICU patients to identify the risks most commonly associated with IC (Bassetti *et al.,* 2010:2).

2.6 Risk factors for developing *Candida* infections

A risk factor is a variable associated with an increased risk for disease or infection. Risk factors for *Candida* infections are most commonly divided into two main groups: host-related factors and healthcare-associated factors (Flanagan & Barnes, 1998:165; Mosby, 2009:1441; Yapar, 2014:102).

The leading host-related factors are: immunosuppression, neutropenia, age and a deteriorating clinical condition due to underlying diseases. Healthcare-associated risk factors include: catheter

use, TPN, surgical interventions, a long stay in the ICU and the use of antimicrobial drugs (Yapar, 2014:101).

Elderly patients are hospitalised more frequently and have a higher mortality rate, longer hospitalisation and increased risk of frailty when compared to younger patients. Conditions typically associated with candidemia are frequently reported in the elderly ICU population with these patients frequently having longer LOS than younger patients. This then resulting in a higher likelihood of IC incidence during hospitalisation (Aminzadeh & Dalziel, 2002:238; Fronczek *et al,* 2018: 245; Muscedere *et al.,* 2011:1985).

Any invasive procedure or apparatus poses a high risk to a patient for developing candidiasis due to invasion of a system. Common risk factors for the development of candidaemia include, but are not limited to (Flanagan & Barnes, 1998:165; Mosby, 2009:1441; Yapar, 2014:102):

- Surgical interventions
- Mechanical ventilation
- Previously received antibiotic treatment
- Neutropenia, solid tumour and haematological malignancies
- Total LOS in hospital
- The presence of central venous catheters
- Prior candidaemia
- Parenteral nutrition
- Chronic renal insufficiency.

Intravascular and urinary tract catheters have been identified as highly significant risk factors for colonisation predisposing patients to infection (Bassetti *et al*., 2010:2; Concia *et al*., 2009:10; Grewe *et al*., 1999:413). In some patients, the portal of entry for colonisation can be traced to intravascular devices, as infection is facilitated by frequent use (Akbari & Kjellerup, 2015:459).

Intravenous catheters, especially when in place for longer than 72 hours, represent an independent risk factor associated with invasive *Candida* infections (Silva *et al*., 2011:289). The development of catheter-related infections is associated with the biofilm formation on the device (Kojic & Darouiche, 2004:255).

Candida biofilms are complex microbial communities that possess unique characteristics (Akbari & Kjellerup, 2015:459). It forms in three different stages, starting when the organisms attach to the surface of the catheter; they then subsequently secrete extracellular polymers and finally form a 3-D structure that surrounds and protects the organisms (Kojic & Darouiche, 2004:255).

Contamination of the catheter surface at the time of insertion can introduce microorganisms into the catheter lumen leading to infections (Silva *et al*., 2011:289).

Total parenteral nutrition is associated with decreased intestinal mucosal barrier function that contributes to atrophy and intestinal ischaemia (Blumberg *et al*., 2001:177; Grewe *et al*., 1999:413). The combination of the invasive device and the hypertonic TPN mixture dramatically increases the risk for IC (Bassetti *et al*., 2010:2; Concia *et al*., 2009:10; Hollenbach, 2008:27).

Surgery and trauma are major risk factors for the development of secondary fungal infections. The critically ill patient is usually immunosuppressed following major surgery, trauma or burns and this predisposes the patient to invasive mycoses (Bessey, 2007:131; Pinhati *et al*., 2016:433).

Abdominal surgery is one of the most important risk factors for candidaemia (Eggimann *et al*., 2011:6). Opening or perforation of the bowel disrupts the mucosal and cutaneous barrier, resulting in contamination of the peritoneum by digestive flora (Pfaller *et al*., 2011:123).

Patients with burns are at an increased risk of fungal infections due to the loss of the skin barrier (Fridkin & Jarvis, 1996:501; Pappas *et al*., 2015:411). These patients are frequently immunosuppressed because of their burns (Bassetti *et al*., 2010:2) and the lack of a skin barrier exposes tissue to pathogens and puts the patient at extremely high risk for colonisation and mycosis (Pfaller *et al*., 2011:123).

Prolonged life support of failing organs combined with the selective pressure of broad-spectrum antibiotics constitutes key risk factors for IC in non-surgical critically ill patients (Kett *et al*., 2011:670). The administration of antimicrobial agents is a major risk factor for development of IC and is especially influenced by the number of agents used as well as the duration of treatment (Bassetti *et al*., 2010:2; Concia *et al*., 2009:10; Hollenbach, 2008:27).

Long-term and high-density colonisation through the suppression of the normal bacterial flora in the GIT has been shown to lead to invasive *Candida* infections (Bassetti *et al*., 2010:244). In another study, heavy antibiotic exposure, with previous use of two or more antibiotics for at least 14 days, was identified as a risk factor for colonisation (Colombo *et al*., 2006:2820).

Candida colonisation has a significant influence on the level of risk for development of candidaemia and risk for infection is more related to the presence or absence of colonisation than

the number of regions colonised (Yapar, 2014:102). The following factors have a significant influence on colonisation and ultimately infection (Flanagan & Barnes, 1998:165; Hollenbach, 2008:27; Mosby, 2009:1441; Yapar, 2014:102):

- Antibiotic treatment
- Immunosuppression
- Patient age
- The presence of any malignancy
- Previous colonisation and the severity of disease
- Gastric acid suppression
- Invasive procedures and devices
- Total parenteral nutrition and hyperglycaemia
- Severe acute pancreatitis
- Neutropenia
- Mechanical ventilation
- Renal failure
- Malnutrition
- Lengthy ICU stay.

Certain risk factors are present in all patients, namely prior abdominal surgery, intravascular surgery, TPN, broad-spectrum antibiotics, immunosuppression, corticosteroid therapy, acute renal failure, diabetes, transplants, haemodialysis and pancreatitis (Hollenbach, 2008:27; Yapar, 2014:102).

There are, however, some specific risk factors that influence ICU patients and predispose them to infections, which include a prolonged stay and *Candida* colonisation (Yapar, 2014:102). Studies have shown that the incidence of IC peaks around day 10 of the ICU stay (Eggimann & Pittet, 2014:1429; Pfaller *et al*., 2011:120; Vincent *et al*., 2009:2329).

2.7 Clinical presentation

Clinical presentation is the constellation of physical signs or symptoms associated with a particular morbid process, the interpretation of which leads to a specific diagnosis. Symptoms of invasive fungal infections are similar to other infections in the ICU (Badiee & Hashemizadeh, 2014:195).

The early clinical manifestations are non-specific with no clinical sign or symptom sufficiently specific to confirm a positive candidiasis diagnosis (Eggimann *et al*., 2011:1). The prognosis also worsens rapidly with delayed initiation of antifungal therapy (Kautzky *et al.,* 2015:132). Invasive candidiasis may involve all tissue and organs and so it may present with virtually all clinical signs and symptoms inherent to the ICU (Badiee & Hashemizadeh, 2014:195; Bassetti *et al*., 2010:2). The following signs may serve as an indication for IC:

- Discrete haemorrhagic or erythematous palpable rash as a clinical manifestation of small vessel vasculitis due to acute disseminated candidiasis
- Large valvar vegetation with a higher frequency of systemic embolic events is seen in *Candida* endocarditis compared to bacterial endocarditis
- Osteomyelitis and nerve root compression syndromes
- Endophthalmitis with choroid- and/or retinal lesions and subsequent mostly unilateral blindness as another complication of untreated candidaemia
- Hepato-splenic candidiasis with pain in the right upper abdominal quadrant, a tender liver, splenomegaly, low-grade fever and elevated serum alkaline phosphatase may lead to the diagnosis of chronic disseminated candidiasis
- Long-lasting unspecific clinical signs not resolving with antibiotic therapy may lead to a relatively rare diagnosis (Hollenbach, 2008:27).

2.8 Diagnosis

Positive diagnosis of IC requires the isolation of *Candida* spp. from at least one blood or sterile fluid culture that indicates that the spp. have gained access to normally sterile sites (Flanagan & Barnes, 1998:167).

The only way to positively confirm candidiasis is by use of a positive fundoscopic examination (Eggimann *et al*. 2011:4). Classical diagnostic methods, such as direct microscopy,

histopathology and culture, exhibit a limited sensitivity to detect IC and their usefulness depends on the possibility of obtaining samples of deep tissues (Tacconelli *et al*., 2016:276).

The key diagnostic tool is conventional blood and tissue cultures from sterile body sites (Eggimann *et al*., 2011:4). Blood cultures, both from a central venous catheter and a peripheral line, remain the cornerstone for diagnosis of candidaemia (Bassetti *et al*., 2010:5). These methods not only allow for diagnosis, but test the cultures for sensitivity to various antifungal agents (Eggimann *et al*., 2011:4).

Unfortunately, the sensitivity reported is not optimal and the time from blood sample collection to the microbiological response is often lengthy (Bassetti *et al*., 2010:3). Species identification and susceptibility testing take several days, resulting in persisting high mortality rates despite the availability of new antifungal agents (Eggimann *et al.*, 2003:696).

Further diagnostic methods include: direct examination and culture testing of tissue that may demonstrate pseudo hyphae of *Candida*, indicating invasive disease (Shareck & Belhumeur, 2011:1012). Traditional culture from sterile sites other than the bloodstream is useful for the diagnosis of IC (Bassetti *et al*., 2010:4). This usually requires tissue sampling, a high risk invasive procedure with generally low diagnostic yield (Eggimann *et al*., 2011:4).

Newer methods for diagnosis of invasive *Candida* infection are being investigated, including serological markers (mannan and β-d-glucan) and real-time polymerase chain reaction (PCR). The use of β-d-glucan is currently being investigated in ICU populations.

Even though the results seem promising, no large prospective studies have been performed and the main problems for the use of β-d-glucan remain high cost and a high rate of false positive results (Bassetti *et al*., 2010:4, Bloos *et al*., 2018:472). Cultures from sites other than blood or normally sterile body fluids are nonspecific and reflect colonisation in most cases, while blood cultures reflect a positive result in a minority of patients with deep-seated candidiasis (Eggimann *et al*., 2011:4).

The conventional identification of *Candida* to the spp. level usually requires one to three days after detection of fungal growth in blood cultures resulting in poor treatment and increased morbidity and mortality (Eggimann *et al*., 2011:4).

The recent development of new laboratory techniques, fluorescent in situ hybridisation (FISH) and matrix-assisted laser desorption ionisation time of flight mass-spectrometry (MALDITOF-MS), significantly helps to reduce the delay to spp. level identification and therefore allows an earlier choice of appropriate antifungal therapy (Eggimann *et al*., 2011:4).

2.9 Approach to antifungal therapy

There is no single strategy that can be considered the most appropriate for the management of candidaemia in the ICU. In fact, different approaches can be chosen and can be judged as the best for a given clinical situation (Eggimann *et al*., 2011:8). Although antifungal treatment has proven to be extremely effective in the treatment of IC, it remains important to remove the IV catheter for effective treatment of the infection (Bassetti *et al*., 2010:4).

The use of anti-fungal treatment for candidiasis in ICUs usually falls into one of four categories (Eggimann *et al*., 2011:8):

- Prophylactic treatment
- Pre-emptive treatment
- Empirical treatment
- Targeted treatment.

Efficacy and efficiency of the agent selected for treatment are extremely important to patient outcomes (Hollenbach, 2008:32) and the four categories will be discussed in detail below.

2.9.1 Prophylaxis

Prophylaxis is defined as the administration of an antifungal agent to a patient with no evidence of a *Candida* infection (Bassetti *et al*., 2010:4). This is done in the hope of preventing future infection. (Eggimann *et al*., 2011:8). Prophylactic measures are taken for disease prevention, as opposed to disease treatment (Allegranzi *et al.,* 2018:507; Bassetti *et al*., 2010:4). Successful prophylactic treatment requires delivery of the antimicrobial agent in effective concentrations (Allegranzi *et al.,* 2018:507).

Although different antifungals can show comparable efficacy in treating candidaemia, their differences in terms of pharmacokinetics and pharmacodynamics remain significant and can affect the clinical outcome of fragile patient populations (Calandra *et al.,* 2016:126).

There are no definitive indications for starting prophylactic antifungal therapy in ICU patients and therefore, doctors retain the responsibility of management of the infection based on patient-specific risk factors and pathophysiology (Kautzky *et al.,* 2015:132; Sardi *et al*., 2013:62). In view of the high mortality associated with invasive candidiasis, prophylaxis for selected patients in the ICU who are at high risk for the disease would appear to be appropriate (Kullberg & Arendrup, 2016:1448).

Although numerous clinical risk factors for IC could be identified, most of them are frequently found in patients requiring intensive care and are therefore of little relevance to start antifungal treatment. Clinical scoring systems such as the *Candida* Colonisation Index by Pittet and colleagues or the four-risk factor-based *Candida* score by León and colleagues are very useful tools to select these patients who would benefit from the administration of systemic antifungal therapy (Eggimann & Pittet, 2014:1429, Kautzky *et al.,* 2015:132; León *et al*. 2006:730).

Scoring systems or predictive rules that combine clinical risk factors and information for *Candida* colonisation have been proposed to guide patient-specific treatment (León *et al*. 2006:730). Surgery, multifocal colonisation, TPN and severe sepsis significantly predicted IC (Eggimann *et al*., 2011:6). By attributing one point to each risk factor, the score for a cut-off value of 2.5 had a sensitivity and specificity of 81% (Eggimann *et al*., 2011:6).

The knowledge of epidemiological data, known risk factors and analysis of local epidemiology of candidaemia in a singular ICU, allows one to determine whether a patient is at low, moderate or high risk of developing this infection. This consequently determines the choice of the most appropriate management strategies (Bassetti *et al*., 2010:4).

Antifungal prophylaxis might be warranted for ICUs with a high rate of IC (Bassetti *et al*., 2010:4). This approach should be restricted to specific subgroups of patients in whom it has been demonstrated to be useful (Eggimann *et al*., 2011:8). The strategy of targeted antifungal prophylaxis is well established and more effective specifically for ICU patients with persistent neutropenia after treatment for haematological malignancies or after bone marrow transplantation. However, the routine use of antifungal prophylaxis in the general ICU setting is discouraged (Bassetti *et al*., 2010:4).

The approach of limiting prophylaxis to a subgroup of patients with the highest risk of candidaemia may help to limit the quantity of antifungals used and delay the emergence of infections due to fluconazole-resistant *Candida* strains, as seen in immunocompromised patients (Bassetti *et al*., 2010:4).

Rapid initiation of appropriate antifungal therapy has been shown to reduce morbidity in patients with candidaemia and confirmed that prophylactic fluconazole administration in ICU patients reduces the rate of *Candida* infection; however, no clear survival advantage was observed (Guery *et al*., 2009:55).

Eggimann and colleagues investigated the utility of prophylaxis with fluconazole (400 mg/day) in a group of high-risk surgical patients (Eggimann *et al*., 2011:6). Only one patient in the fluconazole group developed candidaemia, compared to seven patients (35%) in the placebo group ($p = 0.02$)

and no patients died due to fungal infection in the prophylaxis group compared to four patients (20%) in the placebo group (Eggimann *et al*., 2011:6, Pennisi & Antonelli, 2009:21).

Although no single study has demonstrated a positive impact on mortality, the Infectious Diseases Society of America (IDSA) guidelines concerning treatment of candidiasis suggest that in selected populations of non-neutropenic critically ill patients, prophylaxis for candidiasis must be considered (Pennisi & Antonelli, 2009:21).

The main advantage of prophylaxis is the possibility of reduction in the prevalence of candidaemia (Bassetti *et al*., 2010:4). On the other hand, the disadvantages of fluconazole prophylaxis include possible toxicity and a profound influence on local epidemiology with the emergence of fluconazole-resistant isolates. As a result, the routine use of antifungal prophylaxis in the general ICU setting is discouraged (Eggimann *et al*., 2011:8).

The timely initiation of antifungal treatment is a critical component in the outcome for the patient. Unfortunately, patients with fungal infection often die of complications attributed to the infection, despite antifungal therapy (Badiee & Hashemizadeh, 2014:203).

Many antifungal drugs exhibit marked variability in drug concentration as a result of inconsistent absorption, metabolism, elimination or interaction with concomitant medications. For each of the available antifungal drugs, both preclinical and clinical trials have exhibited a relationship between serum concentrations and treatment efficacy (Calandra *et al.,* 2016:126). Prophylaxis has not been validated for ICU patients and concerns about the selection of less susceptible *Candida* spp. are a serious limitation (Eggimann *et al*., 2011:8). By indiscriminately prescribing, antifungals have promoted a shift to reduced susceptibility (Eggimann *et al*., 2011:6).

The clinical manifestations of fungal infection are not specific, and like other infective diseases, a high degree of suspicion is required for the early diagnosis and optimal management of these infections (Badiee & Hashemizadeh, 2014:195). Ultimately, the application of antifungal prophylaxis should be carefully considered for each patient and the selection should be patient specific (De Waele *et al.,* 2003:130).

2.9.2 Pre-emptive therapy

Pre-emptive therapy is the treatment of individual patients thought to be at high risk of developing candidiasis (Flanagan & Barnes, 1998:170). Pre-emptive therapy is triggered by microbiological or biomarker evidence of fungus without an actual infection (Calandra *et.al,* 2016:126).

This is the approach to therapy by identification of high risk patients and then administration of treatment to these patients in a pre-emptive manner, specifically based on the probability that they will eventually develop a fungal infection due to identified risk factors.

High risk patients are identified by persistent colonisation and other laboratory markers to manage the disease process (Flanagan & Barnes, 1998:171). This results in an overall reduction of unnecessary antifungals used in the ICU, simultaneously preventing delays in therapy for patients who specifically need it (Flanagan & Barnes, 1998:171).

The main concept of a pre-emptive strategy is to identify patients at high risk for developing candidaemia. Pre-emptive therapy is ideal for use in patients where repeated growth of *Candida* is isolated from at least two different sites (colonised patients) (Caggiano *et al.,* 2011:7041) or those with high-risk scores (Eggimann *et al*., 2011:8), and it is the most frequently used therapeutic strategy in ICUs (Flanagan & Barnes, 1998:171; Gong *et al*., 2016:60).

2.9.3 Empirical therapy

Empirical treatment is defined as the administration of antifungals in the presence of persistent and refractory fever (as defined in the 'List of definitions'). It is recommended in critically ill patients with risk factors for IC and no other known cause of fever (Kautzky *et al.,* 2015:132; Sardi *et al*., 2013:62). The fever is typically self-limiting and manifests as acute inflammation (Bassetti *et al*., 2010:4).

Empirical treatment is commonly used in patients who are at high risk for fungal infections, without microbiological testing or confirmation of a fungal infection (Flanagan & Barnes, 1998:171). This includes treatment of patients presenting with signs and symptoms of an infection, but no positive confirmation completed for a fungal infection with blood or tissue cultures (Kautzky *et al.,* 2015:132; Sardi *et al*., 2013:62).

Current IDSA guidelines recognise that it is appropriate to start empirical therapy in neutropenic patients after four days of persistent fever despite antibacterial treatment (Pennisi & Antonelli, 2009:21). Persistently high or rising fever with no response to broad-spectrum antibiotics (Flanagan & Barnes, 1998:171) will require the administration of antifungal treatment.

In candidaemia patients, delaying the empirical treatment of *Candida* bloodstream infections until positive blood culture results have been obtained has been shown to result in increased mortality (Pennisi & Antonelli, 2009:21).

Despite limited evidence, empirical treatment currently relies on the identification of patients with a high documented risk and on the positive predictive value of risk assessment strategies, such

as the *Candida* Colonisation Index, *Candida* score and predictive rules based on combinations of risk factors (Eggimann *et al*., 2011:8).

The interval between exposure to risk factors and development of IC opens a window of about one week for a structured evaluation to identify patients who may truly benefit from early empirical antifungal treatment according to the underlying condition and immune status (Eggimann *et al*., 2011:8).

Until reliable diagnostics become available, it is necessary to analyse epidemiological data to identify patient-related and external risk factors and set up risk stratification systems (Pennisi & Antonelli, 2009:21). The aim of such systems is to identify high-risk patients who may benefit from prophylactic, empirical or pre-emptive treatment strategies (Pennisi & Antonelli, 2009:21).

2.9.4 Definitive therapy

Definitive therapy is used in patients with a microbiological and/or histological diagnosis of candidiasis (Flanagan & Barnes, 1998:171). This is defined as treatment with appropriate antifungal therapy in patients reflecting a positive diagnosis based on blood or tissue culture results. It is species specific, and targeted treatment is applied.

With this approach, treatment can be changed based on the results of species determination and susceptibility testing, and patients who improved clinically might be suitable for step-down oral therapy to complete the course of 14 days (Eggimann *et al*., 2011:8).

The available oral antifungals for step-down therapy are fluconazole, itraconazole, voriconazole and posaconazole (Bassetti *et al*., 2010:4).

The cost of antifungal therapy is always an important factor to be considered and the switch from IV therapy to oral should always be sooner rather than later. Although efficacy and safety should never be compromised, clinicians should be mindful of cost and utilise less expensive antifungals or an oral formulation whenever appropriate (Hollenbach, 2008:32).

2.9.5 Treatment with pharmaceutical agents

Antifungal treatment is divided into three main groups that will be described in detail in the section below. Currently available treatment includes:

- Polyenes

Amphotericin B deoxycholate belongs to the polyene macrolide class of antibiotics (Gallis *et al*., 1990:309). This class also includes lipid complex and nystatin.

- Azoles

Azoles represent one of the primary agents used in the treatment of candidiasis. They are classified as the triazoles and the imidazoles that form the two major categories of azoles (Hollenbach, 2008:30). Fluconazole, voriconazole, parconazole, etaconazole and ravuconazole all belong to this class of antifungal medicines.

- Echinocandins

The echinocandins (anidulafungin, caspofungin and micafungin) are lipopeptide antifungal agents that inhibit the synthesis of β-1,3-D-glucan in the fungal cell wall and exhibit concentration-dependent fungicidal activity against most spp. of *Candida* (Pfaller *et al*., 2011:120). For concentration-dependent fungicidal activity, the rate of antifungal activity will be maximum at the peak concentration in serum. As the antifungal concentration increases, the rate of fungal activity will decrease. Higher doses of the drug will increase the rate of reduction of fungi (Groll *et al*., 1998:500).

There has been a gradual increase in the number of antifungal compounds and classes discovered since the 1990s. These include: polyenes, azoles, echinocandins and purine analogues. Due to the increased availability of antifungal drugs, selection has occurred with consequent resistance of these micro-organisms (Eggimann *et al*., 2011:6).

Doctors retain the option of giving the drugs for prophylaxis, empiric therapy, preventive treatment or while waiting for the disease to be diagnosed, so that there is a degree of excessive exposure to these agents (Eggimann *et al*., 2011:6; Flanagan & Barnes, 1998:170).

2.9.5.1 Polyenes

Amphotericin B is an alternative regime. The agent has played a major role as antifungal therapy for systemic fungal infections since the 1950s, and remains one of the treatments of choice for many conditions. Amphotericin B is isolated as a by-product resulting from the fermentation process of *Streptomyces nodosus*, a soil actinomycete (Gallis *et al*., 1990:308).

Amphotericin B is used as a broad-spectrum fungicidal agent. It is available and effective against both yeast and moulds. *Candida albicans*, *C. tropicalis* and *C. parapsilosis* are commonly susceptible to amphotericin B (Hollenbach, 2008:30).

Candida krusei is intrinsically resistant to fluconazole, and infections caused by this species are strongly associated with prior fluconazole prophylaxis and neutropenia. Studies have also shown that *C. krusei* displays weak susceptibility to Amphotericin B (Pfaller *et al*., 2011:120-127).

Candida lusitaniae, which accounts for 1 to 2% of all candidaemia, is susceptible to azoles, but has a higher intrinsic resistance to amphotericin B (Bassetti *et al*., 2010:4, McCarthy *et al*., 2017:488). Amphotericin B has negligible bioavailability when administered orally. Therefore, it must be applied intravenously in the treatment of systemic fungal infection (Hollenbach, 2008:30).

It emerged as the preferred polyene and exerts antifungal activity by binding to ergosterol in the fungal cell membrane. This disrupts cell permeability and results in rapid cell death (Groll *et al*., 1998:343). It remains the broadest-spectrum antifungal agent available, with activity against many clinically relevant yeasts and moulds. It is particularly useful in refractory infections, because higher doses can be administered (Hollenbach, 2008:30, McCarthy *et al*., 2017:488).

Invasive candidiasis is routinely treated with amphotericin B. Disseminated candidiasis may be successfully treated with doses of 15 to 30 mg/d for one week (Gallis *et al*., 1990:318) and when conclusive data about the infectious *Candida* spp. are lacking (Hollenbach, 2008:32). High costs and availability of alternative antifungal classes, such as azoles and echinocandins, limit their use (McCarthy *et al*., 2017:488).

2.9.5.2 Azoles

Azole agents exert their antifungal activity by blocking the demethylation of lanosterol, thereby inhibiting ergosterol synthesis. The newer, expanded-spectrum triazoles have been shown to have antifungal activity against a wide spectrum of moulds, as well as enhanced activity against *Candida* species and other yeasts (Bersani *et al*., 2019:375). Fluconazole is a key antifungal compound in the treatment of IC, and has activity against many *Candida* spp. (Hollenbach, 2008:30), while voriconazole can be indicated as step-down therapy for C. *krusei* or voriconazole-susceptible *C. glabrata* (Bassetti *et al*., 2010:4).

For stable patients with *C. albicans* or other fluconazole-susceptible strains, fluconazole is the drug of choice. Importantly, fluconazole is the preferred treatment for *C. parapsilosis*, since resistance to echinocandins has been reported (Bassetti *et al*., 2010:4). The loading dose for fluconazole should be as high as twice the daily dose (Hollenbach, 2008:30).

Fluconazole can either be administered orally with bioavailability unaffected by gastric pH or via IV routes, depending on the patient-specific requirements. The serum concentrations are approximately the same when given orally or intravenously (Hollenbach, 2008:30).

Among the non-*albicans* spp., *C. tropicalis* and *C. parapsilosis* are both generally susceptible to azoles. However, *C. tropicalis* is less susceptible to fluconazole than is *C. albicans*. *Candida*

glabrata is intrinsically more resistant to antifungal agents, particularly to fluconazole (Trevor *et al*., 2005:404-411).

Intravenous fluconazole is often the treatment of choice for a systemic *Candida* infection, switching to oral therapy until the signs and symptoms of the infection disappear (Trevor *et al*., 2005:404-411).

2.9.5.3 Echinocandins

The echinocandins represent the newest class of antifungal drugs with caspofungin released in 2001. The mechanism of activity of the echinocandins is the inhibition of the production of (1r3)-β-d-glucan, an essential component in the fungal cell wall (Groll *et al*., 1998:500). The spectrum of activity is therefore limited to pathogens that rely on these glucan polymers and is less broad than the spectrums of the polyene or azole agents.

Based on clinical and experimental data, the available echinocandins, caspofungin, micafungin and anidulafungin, may be recognised as interchangeable for IC, but costs and availability will mainly affect their individual use (Hollenbach, 2008:30; Tacconelli *et al*., 2016:276).

The agents recommended for initial treatment of candidaemia in critically ill patients include echinocandins and amphotericin B, but the choice regarding approach to treatment is crucial (Trevor *et al*., 2005:404-411).

All echinocandins have a poor bioavailability and are administered intravenously. They are currently regarded as primary treatments for invasive candidiasis or candidaemia in non-neutropenic and neutropenic patients with moderate to severe illness or recent exposure to azoles (Muilwijk *et al.,* 2014:3294).

Echinocandins should be used as soon as critically ill patients with negative fungal cultures become unstable. This is especially important for neutropenic patients, patients with a fever of unknown origin or patients who are septic (Tacconelli *et al*., 2016:276). This is because the echinocandins have significantly fewer drug-related adverse events than amphotericin B, and therefore has been licensed for first-line therapy of oesophageal candidiasis, candidaemia, intra-abdominal abscess, peritonitis and pleural effusion due to *Candida* (Hollenbach, 2008:30).

The interest in combining antifungal therapy to increase the spectrum and availability of antifungal agents has increased since this will result in more potent antifungal activity. By combining different modes of action, you might expect broader-spectrum antifungal coverage and a limited impact on the development of drug resistance (Hollenbach, 2008:31).

Single agents are usually less effective than combination regimens, and therefore appropriate statistically powered evidence-based studies seem to justify the use of antifungal combination therapies (Hollenbach, 2008:32).

The differences in drug-related toxicity are significant and the possibility of drug interactions is also important in critically ill patients who receive numerous treatments. The efficacy of the treatment should be assessed by the documentation of blood cultures returning sterile (Bassetti *et al*., 2010:6).

The first section of the literature focused on the epidemiology, risk factors, pathogenesis, clinical presentation, diagnosis and treatment of *Candida* infections. The focus will now shift to antibiotics, with a specific focus on the risk associated with developing secondary *Candida* infections due to exposure.

2.10 Antibiotics

"*If I give my patients too many antibiotics — say I am in a hospital, working in a ward and I create resistant organisms in my patients, those organisms can then be spread to other patients on the ward.*

My patients can leave the hospital and take those organisms to other hospitals. So, I as one individual, with my prescribing pen in one hospital, can create a problem for a very large group of individuals who I've never even met. …

Antibiotics are in a way a public resource, so we have a responsibility … to steward that resource effectively, much like we do natural resources.

There were a lot of people who have said that we really should think about antibiotics the way we think about the environment.

We all share the air we breathe. If I pollute the air, it has a negative impact on you, just like antibiotics.

If I misuse antibiotics, it can have a negative impact on you."

- Dr Arjun Srinivasan, On Antibiotic stewardship, 2013

Antibiotics are known to be the most successful group of drugs ever developed for the treatment of human patients, ultimately resulting in improved health (Martinez, 2009:2893). This group of drugs revolutionised the approach towards the treatment of bacterial infections with an extraordinary impact on previously known fatal illnesses (Trevor *et al*., 2005:404-411).

Seen initially as truly miraculous drugs, access to the first available systemic antibiotics was not immediately available to the public. In fact, these drugs were scarce and very expensive, and were initially reserved for use only by the military during World War II (Alanis, 2005:697).

Progression resulted in antibiotic discovery, rapid development of simplified manufacturing processes and newer formulations. This allowed increased, widespread application of antimicrobial agents, making antibiotics easily accessible to all (Alanis, 2005:698).

It is extremely important to follow the correct rationale during selection of antibiotic treatment by correctly identifying the infective organism, the site and severity of the infection, and determining the co-morbid diseases. It is important to establish the immune status of the patients as well as renal and hepatic function. Another aspect of effective antibiotic management is effective administration – this includes dose, duration and route of administration (Trevor *et al*., 2005:404-411).

2.10.1 Antibiotics – a history

The word antibiotic was first used as a noun by Selman Waksman in 1941 to describe any small molecule made by a microbe that antagonises the growth of other microbes (Clardy & Walsh, 2004:829). The discovery of the antibiotics and antibacterial agents revolutionised the treatment of infectious bacterial diseases that used to kill millions of people worldwide (Bbosa *et al.*, 2014:423).

From 1945 to 1955, the development of penicillin, produced by a fungus, along with streptomycin, chloramphenicol and tetracycline, produced by soil bacteria, ushered in the antibiotic age (Clardy & Walsh, 2004:829).

The antibiotic age is the period when the entire antibiotics/antibacterial drug spectra were discovered and almost all the bacterial infections were treatable with these drugs (Bbosa *et al.*, 2014:423). In this period, bacterial infections and diseases were considered the diseases of the past (Hollenbach, 2008:32).

The age of antimicrobial therapy began with the production of penicillin in 1941 to the discovery of nalidixic acid, the progenitor of the fluoroquinolone antibiotics in 1962 (Fischbach & Walsh, 2009:1093). Currently, this period has been extended from 1940 to the 1990s due to the discovery of newer antibiotics, mainly synthetic (Bbosa *et al.*, 2014:423).

During the period of two decades, almost all antibacterial spectra with different generations such as *β*-lactams, tetracyclines, chloramphenicol, aminoglycosides, macrolides, glycopeptides, streptogramins and quinolones with different mechanisms of action on bacteria were introduced in clinical practice (Fischbach & Walsh, 2009:1093).

New antibiotics were then produced by chemical, semi-synthetic or synthetic modifications of pre-existing antibiotics. These modifications produced new generations of antimicrobial agents, with improved efficacy and a broader spectrum of activity (Bbosa *et al.*, 2014:423).

Antibiotics do not look like the familiar original molecules and usually do not even resemble each other, but despite these differences, they are assembled through enzyme catalysed reactions (Clardy & Walsh, 2004:829).

Antibiotics are assembled from the same types of building blocks, for instance: penicillin is derived from a tripeptide of three amino acids, two of which are proteinogenic and one of which is an intermediate in lysine metabolism (Clardy & Walsh, 2004:829).

The evolutionary history of an antibiotic should be a wonderful model for the evolution of multigenic traits, but is complicated by lineage and function (Clardy & Walsh, 2004:829).

2.10.2 Antibiotics – an overview

Antibiotic treatment has revolutionised medical care in a number of ways: infections that were life threatening or gave patients lifelong morbidity can now be controlled and eradicated completely (Martinez, 2009:2893). It is used to treat infection and can have either bactericidal or bacteriostatic effects on pathogens (Davis, 1987:341). Some of them can do both, depending on the concentration of the antibiotic and the bacteria on which they act (Pankey & Sabath, 2004:864).

When an antimicrobial agent is bactericidal, it is lethal and results in the death of the bacteria; while displaying bacteriostatic characteristics, the antibiotic mainly impairs the growth of the bacteria and inhibits the infection (Walsh, 2000:775).

There are several classes of antibiotics, each with unique characteristics and specified applications (Bbosa *et al.*, 2014:414). The different antibiotics have various targets on the bacteria, including cell wall and cell membranes, ribosomes, nucleic acids, bacterial cellular metabolism and bacterial cellular enzymes (Kohanski *et al*., 2007:797).

Antibacterial drug target interactions are well studied and predominantly fall into three classes: inhibition of deoxyribonucleic acid (DNA) replication and repair, inhibition of protein synthesis, and inhibition of cell-wall turnover (Walsh, 2000:775).

The different mechanisms for inhibition as well as the destruction of bacteria include:

- Inhibition of cell wall synthesis such as beta lactams
- Disruption of cell-membrane function

- Inhibition of protein synthesis in both ribosomal subunits (50S and 30S)
- Inhibition of nucleic acid synthesis both the DNA- and ribonucleic acid (RNA) synthesis
- Action as antimetabolites (Bbosa *et al.*, 2014:414).

The differences in the bacteria and mammalian cells, especially the structural and metabolic differences, enable the antibiotics to cause selective toxicity to the bacterial organisms without causing any damage to the host cells (Frost, 2007:51).

Bacterial cells have a cell wall surrounding the cell membrane, which consists of sheets of mucopeptide, a three-dimensional matrix, a mix of polysaccharide and peptide components (Navarre & Schneewind, 1999:203).

There are two main varieties of bacterial cell walls that are differentiated by the process of Gram staining (Beveridge, 1999:4725). Cells stained purple by the process are termed Gram-positive, while cells that are not coloured are termed Gram-negative (Frost, 2007:51).

Gram-negative bacteria have an additional coat of lipid in addition to the murein, which provides additional defence against both the body's immune system and antibiotics (Trevor *et al*., 2005:404).

Many antibiotics will act preferentially against either Gram-negative or Gram-positive bacteria (Beveridge, 1999:4725). Beta-lactams, which include carbapenems, penicillin, cephalosporins and mono-bactams, work by binding to various enzymes in the cell wall, and prevent the successful cross linking required for the three-dimensional matrix of the cell wall (Droege *et al.,* 2016:25).

Interaction with peptidoglycan building blocks interferes with cell wall synthesis and induces lysis, weakening of the cell wall and death (Reynolds*,* 1989:943). The loss of structure in the cell wall results in the death of the cells (Droege *et al.,* 2016:25).

Cephalosporins can be sub-categorised further into four generations as a guide to their relative activity against different bacteria (Droege *et al.,* 2016:26). Earlier generation cephalosporin antibiotics are more active against Gram-positive than Gram-negative bacteria (Livermore, 2012:128). Later generation cephalosporins have greater activity against Gram-negative bacteria (Livermore, 2012:128).

Bacterial cell walls grow by adding chains of five amino acids onto the peptidoglycan matrix (Frost, 2007:511). Glycopeptides bind to the cell wall structure to prevent the corporation of N-

acetylglucosamine and N-acetylmuramic acid into the cell wall's peptidoglycan matrix (Frost, 2007:51).

This produces a rigid three-dimensional structure for the cell wall and the action of glycopeptides fatally weakens the bacterial cell (Frost, 2007:51). Some bacteria, such as *Mycoplasma,* lack cell walls altogether and consequently beta-lactam antibiotics have no target molecules to act on and are ineffective (Frost, 2007:51).

While eukaryotic cells arrange their DNA in many chromosomes surrounded by a membrane, most bacterial DNA exist as a loop in the bacterial cytoplasm (Navarre & Schneewind, 1999:203).

Bacteria can also have DNA in separate loops, known as plasmids, which can be passed between bacterial cells (Navarre & Schneewind, 1999:203). Transfer of these plasmids can cause problems with the genes that encode proteins that generate antibiotic resistance (Frost, 2007:51).

Quinolones target DNA replication and repair by binding DNA gyrase complexed with DNA, which drives double-strand DNA break formation and cell death (Drlica & Zhao, 1997:377).

Antibiotics acting on nucleic acids and DNA include:

- Trimethoprim and sulphonamides – act by inhibiting metabolic pathway that produces thymidine, one of the components of DNA (Kohanski *et al.,* 2007:797)

- Quinolones – inhibit DNA gyrase and DNA topoisomerases, key enzymes in the control of DNA replication during cell division. In the absence of the control that these enzymes provide, the DNA that is produced lacks the proper shape to function correctly (Kohanski *et al.,* 2007:798).

Bacteriostatic drugs predominantly inhibit ribosome function, targeting both the 30S (tetracycline- and aminoglycoside family) and 50S (macrolide family and chloramphenicol) ribosome subunits (Poehlsgaard & Douthwaite, 2005:870).

The aminoglycoside family of drugs is the only class of ribosome inhibitors known to cause protein mistranslation (Weisblum & Davies, 1968:493).

There are several different antibiotics that work by reducing the action of the ribosome, including:

- Aminoglycosides that bind to the smaller half of the ribosome (the 30S sub-unit) and prevent the formation of the initiation complex; the first step in developing a new protein

- Tetracyclines block ribosomal acceptor sites from accepting transfer RNA, the carrier molecule that transports amino acids

- Chloramphenicol binds to the larger half of the ribosome and prevents the linking of amino acids by peptide bonds

- Macrolides bind to the 50S sub-unit that blocks the translocation step of protein biosynthesis, essentially preventing the ribosome from functioning

- Linezolid binds to the 50S sub-unit, preventing the two sub-units of the ribosomes from functioning together (Chopra & Roberts, 2001:232; Frost, 2007:51; Poehlsgaard & Douthwaite, 2005:870; Weisblum & Davies, 1968:493).

The different classes of antibiotics/antibacterial agents that are commonly used in clinical practice to treat bacterial infections are described in more detail in Annexure G.

2.10.3 Antibiotics – applications

Antibiotics continue to be administered in human, animal and plant infections in an abundant fashion and due to the effective application in various fields of use, the practice continues to expand exponentially (Furaro *et al.,* 2018:2660; Martinez, 2009:2893).

In the last 60 years, major improvements in the early recognition and treatment of infectious diseases have resulted in an extraordinary reduction in the morbidity and mortality associated with infections (Alanis, 2005:697), but also influenced the rate of application and distribution of antibiotics resulting in frequent use worldwide (Istúriz & Carbon, 2000:395).

This has resulted in antibiotics released in large amounts in natural ecosystems causing antibiotic pollution (Martinez, 2009:2893). The success of antibiotics was largely attributed to the rapid development of safe and effective agents able to eliminate infection in the patients treated (Alanis, 2005:697); this, however, has come to a complete standstill in the past five years, with no new antibiotic drug developed or produced (Istúriz & Carbon, 2000:395).

Manufacturer dosage indication (MDI) for antibiotics refers to the authorised indication and specific dosage of that drug for treating a disease as per development research results. When there is enough evidence to approve the medicine for the specific indication, that becomes the approved dosage and indication (Thompson & Webb, 2014:866).

Studies have shown that the use of risk adjustment may be needed to overcome challenges related to application of MDI (Momattin *et al*., 2018:312), as it is important to view the patient as

individual. However, the fact remains that an estimated 20 – 50% of antibiotic use is inappropriate or unnecessary in acute care hospitals (Momattin *et al*., 2018:312).

This phenomenon has a direct negative impact on patients, and ultimately the community, and therefore appropriate application of MDI should be benchmarked and monitored to prevent misuse (Momattin *et al.,* 2018:313).

Bacteria can evolve and develop antibiotic resistance through several mechanisms, including alteration by mutations of the antibiotic target, changes in cell permeability and efflux, and horizontal transfer of resistance genes (Rodríguez-Rojas *et al*., 2013:293).

The increasingly rapid emergence and dissemination of antimicrobial-resistant bacteria have become a world-wide problem with an exceedingly unpredictable spectrum of consequences (Martinez, 2009:2893), yet these agents remain one of the most frequently prescribed classes of drugs in the ICU (Ntagiopoulos *et al*., 2007:363).

Increased bacterial resistance is exceedingly evident, with an obvious drop in the number of antibiotics discovered and approved each year (Momattin *et al*., 2018:312). Ultimately, the misuse of antibiotics leads to an increase in antibiotic resistance and additional healthcare costs (Ibrahim & Polk, 2014:195).

2.10.4 Effects and side effects

When antibiotics were first introduced into medicine approximately 70 years ago, the rationale of dosing was relatively simple: to achieve a therapeutic dose at the infected site that was high enough to clear the bacterial infection without having a severe toxic effect in the patient (Andersson & Hughes, 2011:1).

Initial research on bacterial susceptibility and antibiotic dosing introduced one of the central concepts of the field: the minimum inhibitory concentration (MIC), which is defined as the lowest concentration of drug that, under established in-vitro conditions, inhibits visible growth of a target bacterial population (Mouton, 2012:45).

Antibiotic susceptibility is determined by the relationship between MIC and achievable antibiotic concentration. Minimum inhibitory concentration is the lowest concentration required to irreversibly inhibit 99.9% bacterial growth (Droege *et al.,* 2016:22; Pankey & Sabath*,* 2004:864). If the MIC is lower than the antibiotic concentration achieved with a safe dose, the pathogen is considered susceptible (Furaro *et al.,* 2018:2660).

The basic rationale of antibiotic dosing is to maintain an antibiotic concentration that is higher than the MIC in the relevant body compartments for long enough to clear the infection. This was then adapted to suit the characteristics of different drug classes (Andersson & Hughes, 2011:1).

If a bacterial population encounters antibiotic concentrations below the MIC, the chances of high and low level antibiotic resistance development are increased. Furthermore, selection of antibiotic resistance increases the proportion of mutant strain in the population, raising the probability of resistance even to non-related antibiotics (Rodríguez-Rojas *et al*., 2013:295), indicating that the inappropriate use of antibiotic agents increases the risk for resistance immensely.

The spectrum of antibiotic use has evolved dramatically, resulting in an increased consumption of broader spectrum antibiotics, while the use of specified agents decreased. Furthermore, there were no official restrictions or guidelines for antibacterial prophylaxis and therapy until recently, thereby limiting the management of antibiotics (Ntagiopoulos *et al*., 2007:363).

The relationship between antibiotic use and resistance is most evident when resistance is due to mutations selected during therapy, resulting in clinical failure (Alanis, 2005:698). This is evident in many strains of bacteria and in multiple cases displaying multi-resistance to several therapeutic agents, thereby rendering antibiotics ineffective as a treatment of choice for the specific bacterial infections (Alanis, 2005:698).

When antibiotics are used clinically, the primary goal is to achieve the highest possible non-toxic concentration to obtain the highest cure rates and prevent the development of resistance in the host (Andersson & Hughes, 2011:9).

Side effects vary from typical and frequent symptoms to those that are severe, rare and occasionally life-threatening (Bbosa *et al.*, 2014:414). General side effects of these agents include, but are not limited to: hypersensitivity, gastrointestinal (GI) side effects, headache, fever, candidiasis, skin reactions, central nervous system (CNS) effects, renal influence and haemolytic effects. When administering broad spectrum antibiotics, *Candida* super-infections may be experienced (Trevor *et al*., 2005:404-411).

Gastrointestinal effects are common with macrolide antibiotics (Droege *et al.,* 2016:25). Rifampicin can colour body secretions orange or red, which may prove distressing and patients should be advised before treatment commences that this is usual and harmless (Frost, 2007:51).

Bone marrow toxicity, resulting in the failure of the body to produce new erythrocytes, leucocytes and platelets may occur with chloramphenicol. This limits a very potent antibiotic to life-threatening infections when used systemically (Trevor *et al*., 2005:411). Sulphonamides can

produce haematological side effects as well as renal impairment and as a result their use has declined significantly (Frost, 2007:51).

The results of interactions between antibiotics and other drugs can range from trivial to severe (Alanis, 2005:698), for example: rifampicin induces hepatic enzymes, resulting in a faster rate of metabolism of drugs as diverse as oral contraceptives, anti-epileptics, anticoagulants, other antibiotics, cardiac medicines, corticosteroids and medication for diabetes (Alanis, 2005:698).

Other antibiotics can inhibit hepatic enzymes (Alanis, 2005:698). Ciprofloxacin and erythromycin, for example, can inhibit the metabolism of theophylline, resulting in the risk of theophylline toxicity.

The potential for drug interactions should be considered when prescribing any drug for a patient who could be taking another drug (Trevor *et al*., 2005:404-411).

2.10.5 Antibiotics and *Candida* infections

Despite their abundance in nature, very little is known about the interactions of *Candida* with bacteria within a microbiome (Kalan & Grice, 2018:247; Klotz *et al*., 2007:370; Uhl *et al*., 2003:2678). *Candida* is commensal and therefore it is assumed that colonisation is controlled by the combined effort of the host immune system and other microorganisms present in the host (Uhl *et al*., 2003:2678).

Inhibitory factors, normally secreted by bacteria, suppress filamentous growth, keeping *Candida* in check (Kalan & Grice, 2018:247; Uhl *et al*., 2003:2678). A very large number of microorganisms are ever-present in the host resulting in a multitude of interactions and fierce competition for space and resources (Klotz *et al*., 2007:370; Wisplinghoff *et al*., 2004:317).

Numerous physical and chemical interactions have been identified between *Candida* and bacteria (Klotz *et al*., 2007:370; Wisplinghoff *et al*., 2004:317). These physical interactions of *Pseudomonas aeruginosa* and *Candida* are antagonistic (Hogan & Kolter, 2002:2232). *Pseudomonas aeruginosa* can attach to and kill the hyphal-filamentous form of *Candida yet* has no effect on the yeast form (Hogan & Kolter, 2002:2232); whereas *Streptococcus gordonii* and *Staphylococcus epidermidis* appear to be able to attach to both the yeast and hyphal forms of *Candida* (Bamford *et al*., 2009:3704).

Specific molecules from a variety of bacteria including Pseudomonas have been shown to interrupt *Candida*'s ability to transition between morphological forms and lock *Candida* into the yeast form, inhibiting hyphal formation (Boon *et al*., 2008:35). Alternatively, *Streptococcus gordonii* appears to enhance hyphal formation (Bamford *et al*., 2009:3704).

These conflicting effects demonstrate the complex relationships involved in a shared microbiome. When antibacterial therapy is applied, it alters the microbiota (Eggimann *et al*., 2003:691), leading to selective pressure on normal flora, favouring the growth of pathogens (Ntagiopoulos *et al*., 2007:360). In hospitals where prophylactic antibiotics are routinely used, the incidence of *Candida*-related infections is increased (Gloor *et al*., 2001:595).

The depletion of normal bacterial flora allows fungi to proliferate, subsequently colonise and cause infection (Karabinis *et al*., 1998:430). Antibiotics promote fungal overgrowth at the expense of normal bacterial flora (Flanagan & Barnes, 1998:165; Mosby, 2009:1441; Yapar, 2014:102), increasing the prevalence of *Candida* infections (Horn *et al*., 2009:1703).

2.11 Length of stay

Length of stay is the time measured from the date of admission into the ICU until the date of release from the ICU. Length of stay is usually measured in days (Miller-Keane & O'Toole, 2005).

There is a strong relation between presence of infection and length of ICU stay (Vincent *et al*., 2009:2329). Patients colonised or infected with *Candida* have a longer stay both in the ICU and in the hospital than non-colonised, non-infected patients (Olaechea *et al*., 2004:328).

2.12 The relationship between length of stay, antibiotic exposure and secondary *Candida* infections

"Critical illness leading to a prolonged length of stay in an ICU is associate with significant mortality and resource utilisation. Length of stay significantly impacts mortality as this is associated with inappropriate antibiotic use. Effects to decrease rates of inappropriate antibiotic therapy may serve to improve hospital resources use and result in shorted overall hospital stays."

- Andrew Shorr, On Critical Care Medicine, 2011

The prevalence of *Candida*, especially in the ICU, is staggering (Klotz *et al*., 2007:370; Richardson & Naglik, 2018:43; Wisplinghoff *et al*., 2004:317). *Candida* is the fourth most common organism involved in nosocomial blood stream infections and the third most commonly isolated organism of catheter-related infections (Klotz *et al*., 2007:370; Wisplinghoff *et al*., 2004:317).

Mortality of these infections, particularly when involving the bloodstream, can reach as high as 35 to 40% (Klotz *et al*., 2007:370; Richardson *et al.*, 2018:13). This is usually associated with significant morbidity, which is reflected by a long hospital stay, ranging between one and several weeks (Leroy *et al*., 2009:1616).

In case-control studies in which patients are matched at the time of ICU admission according to underlying disease, severity of illness and diagnosis, it is assumed that a prolonged hospital stay is due to nosocomial infection (Jacobs *et al.*, 2015:92).

The relationship between LOS and colonisation is linear (Caggiano *et al*., 2011:7041; Pittet *et al*., 1994:756), meaning that the longer the patient stays in the ICU, the higher the risk of IC (Bessey, 2007:131; Pinhati *et al*., 2016:433). The results suggest that a patient with a longer stay in the ICU is more likely to develop IC (Olaechea *et al*., 2004:328).

This may be attributed to prolonged exposure to invasive interventions (Yapar, 2014:102). Predisposing factors for IC include: multiple antibiotic exposures, immunosuppressive therapy, diabetes mellitus and a long LOS (Jacobs *et al.*, 2015:92). Nearly half of all fungal infections are manifested among surgical ICU patients (Manolakaki *et al.,* 2010:367).

The presence of such risk factors has been advocated as a trigger for the empiric treatment of potentially antibiotic-resistant bacteria and, when appropriate, fungal pathogens (Pennisi & Antonelli, 2009:21).

Colonisation is a proven prerequisite for invasive infection (Jacobs *et al.*, 2015:92). *Candida* colonisation develops in up to 80% of critically ill patients staying more than one week in the ICU, whereas IC is documented in only 50% (Eggimann & Pittet, 2014:302).

Jacobs *et al*. (2015:87) found patients with *Candida* colonisation to have a significant increase in ICU stay and hospitalisation, where LOS increases significantly in the ICU and overall hospitalisation for patients colonised.

The study also indicated that an ICU stay was prolonged by a median of six and 13 days and hospital stay by a median of nine and 15 days in colonised patients (Olaechea *et al*., 2004:328). Prolonged hospitalisation has been calculated using multivariate models (Olaechea *et al*., 2004:328). One such study was reported by Rentz and fellow researchers who demonstrated that patients with candidaemia had an additional 34 days of hospitalisation compared to patients without candidaemia (Rentz & Halpern, 1998:788). In the study of Wey *et al*. (1988:2649), candidaemia prolonged hospitalisation by 30 days in comparison with control groups.

A retrospective study from the United States, which was based on the attributable mortality, LOS and hospital charges related to candidaemia, found that candidaemia was associated with a 14.5% increase in mortality, a mean 10.1-day increase in LOS, thereby leading to a rise in the total expense (Giri & Kindo, 2012:270).

There is an increased prevalence of critical care service utilisation by older individuals together with an increased number of older patients being admitted to ICUs globally (Muscedere *et al.,* 2011:1985). Considering their diminished resilience and greater vulnerability, this results in a higher likelihood of an extended LOS (Muscedere *et al.,* 2011:1985).

Candida bloodstream infections lead to prolonged hospital stays, significant costs and high mortality (Jacobs *et al*., 2015:87). Prompt diagnosis is critical because patients treated more than 48 hours after diagnosis have a lower probability of survival (Vincent *et al*., 2009:2329). The impact of candidaemia on excess mortality, increased LOS and the burden of cost of hospitalisation underscore the need for improved means of prevention and treatment of candidaemia (Giri & Kindo, 2012:270; Jacobs *et al.*, 2015:92).

2.13 Chapter summary

In Chapter 2, the literature review was completed; the chapter introduced the literature used to form this study. A complete review of topic-related literature from reliable sources was completed to outline the clinical considerations related to *Candida*, antibiotics, LOS and the relationship between these three entities. From the literature, it was confirmed that there is a linear relationship between antibiotic exposure and the prevalence of *Candida* infections, which is exasperated in the event of a lengthy ICU stay and extended exposure to antibiotic treatment.

CHAPTER 3: DATA ANALYSIS AND RESULTS

3.1 Introduction

Chapter 3 consists of the results and discussions of the study's empirical investigation. In order to determine the prevalence of secondary *Candida* infections in the specific ICU and to investigate antibiotic exposure as a risk factor for secondary *Candida* infections, the researcher reports and reviews the results of the retrospective data captured from the study sample.

Descriptive statistics, confidence intervals, normality tests and plots, requires a case processing summary, which informs the user of all data that can be used in the analysis. The processing summary is very useful in the confirmation of valid cases to include within the data analysis and identify any data that must be excluded.

The descriptive part of the analysis is an exploration with the purpose to get a clear overview of the data and the distribution of the variables by diagrams, tables and basic statistics, which includes comparisons between the study population, hereafter known as the as the cases group, and a control group.

Descriptive statistics are used to assess associations and, in this case, explore correlations between antibiotic exposure, LOS and prevalence of *Candida* infections in the target population. The chi-square test is used to determine whether an association exists between these categorical variables, with a *p*-value indicating that the difference between the variables is not by chance, but in fact significant.

In addition, the Cramér's *V* statistic tests the practical significance of identified associations with any value of $V \geq 0.5$ is defined as practically significant.

The statistical analysis using Cohen's effect size is used to calculate the strength of the different correlations and identify significant correlations between the variables. The results describe the practical significance of the correlations and if $d \geq 0.5$, the correlation is accepted as a medium effect with practical significance.

The main goal of the data analysis is an in-depth investigation of the correlation between antibiotic exposure and the prevalence of secondary *Candida* infections in the entire population. It also enables the researcher to reach the defined research objectives as documented in section 1.4.1.1. The chapter concludes with a summary.

3.2 Results and analysis

The topics in this chapter are arranged to form the best picture and patterns in the population and to compare the frequencies between the cases group and the control group. This supports the researcher to investigate the prevalence of fungal infections in the specific ICU and assess antibiotic exposure as a risk factor for secondary *Candida* infections via the analysis of illustrations and descriptive statistics.

3.2.1 Study population

The target population included all patients of the specific ICU, admitted and treated during the specified timeframe of the research study. A total of 440 patients were treated within the ICU during the specified timeframe. However, as all available data were subject to the exclusion criteria defined in section 1.6.2.1, a total of 238 cases were excluded from the study. A final study population of 202 cases were confirmed to comply with the specific inclusion criteria defined in section 1.6.1.

The research design further required inclusion of a cases group (who received antifungal treatment) and a control group (who did not receive antifungal treatment) to enable the researcher to associate certain characteristics with adherence or non-adherence.

This approach was followed effectively and determined the frequency with which associations occurred in the study population. Furthermore, enabling data comparison to analyse the significance of correlations within the population. An explanatory analysis of the study population is depicted in table 3.1.

Table 3-1: Study population: Cases group vs control group

Cases group (1) control group (2)					
		Frequency	**Percentage**	**Valid percentage**	**Cumulative percentage**
Valid	Case group	95	47.0	47.0	47.0
	Control group	107	53.0	53.0	100.0
	Total	202	100.0	100.0	

All patients treated for a *Candida* infection were included within the cases group and all patients from the same setting, not treated for a *Candida* infection, were included in the control group. The cases group consisted of 95 participants and the control group included 107 participants. The validity of cases included in the study was supported by the case processing summary.

3.2.1.1 Case processing summary

The case processing summary informs the user of any data that the SPSS® programme could not use in the analysis. The "Explore" function in SPSS® is useful to deeply investigate this single numeric variable, with or without a categorical grouping variable. This is usually due to missing values; the reason for exclusion of an observation from the analysis is included in the summary, represented by the 'N'. This includes the percentages (%) of cases falling into each category. The case processing summary for this study is recorded in table 3-2.

Table 3-2: Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percentage	N	Percentage	N	Percentage
Age	202	100.0%	0	0.0%	202	100.0%

The SPSS® programme did not find any data to be excluded from the case processing summary, confirming that there were no missing values within the study population.

3.2.2 Diagnosis

There was a well-balanced distribution between the number of participants included in the cases and control groups, with the number of participants in the cases group representing 47.0% and the control group representing 53.0% of the total population.

The diagnosis analysis ultimately highlighted the high prevalence of *Candida* within this setting, with almost half of the total population presenting with a *Candida* infection during the study period. This data ultimately formed the basis for the investigation of antibiotic exposure as a risk factor for secondary *Candida* infections in this ICU. The balanced distribution between the groups effectively ensured validity of comparisons and conclusions.

3.3 Antibiotic use

As per section 2.10 of the literature review, it was found that antibiotics promote fungal overgrowth at the expense of normal bacterial flora, increasing the prevalence of *Candida* infections and are therefore considered a major risk factor for prevalence of *Candida* infections. Antibiotic use within the population, which include the cases and control groups, is recorded in Table 3-3.

Table 3-3: Antibiotic use

Antibiotic use					
		Frequency	**Percentage**	**Valid percentage**	**Cumulative percentage**
Valid	No	3	1.5	1.5	1.5
	Yes	199	98.5	98.5	100.0
	Total	202	100.0	100.0	

Antibiotic use was present in most of the participants with 98.5% of participants receiving antibiotic treatment during the study period. A mere 1.5% of participants received no antibiotic treatment while in the ICU during the same period.

Antibiotic administration was evident in the majority of the population and as seen in table 3-1, a clear statistical significance between antibiotic use and the prevalence of *Candida* infection existed, with almost half of the population treated with antifungal therapy. Further analysis of antibiotic administration patterns was required to understand the correlation between antibiotic administration and prevalence of *Candida* infections.

3.3.1.1 Single versus multiple antibiotic administration

The risk for fungal infection increases exponentially with each class of antibiotic added during treatment (refer to section 2.2). Figure 3-1 illustrates the high prevalence of administration of multiple antibiotics within the population.

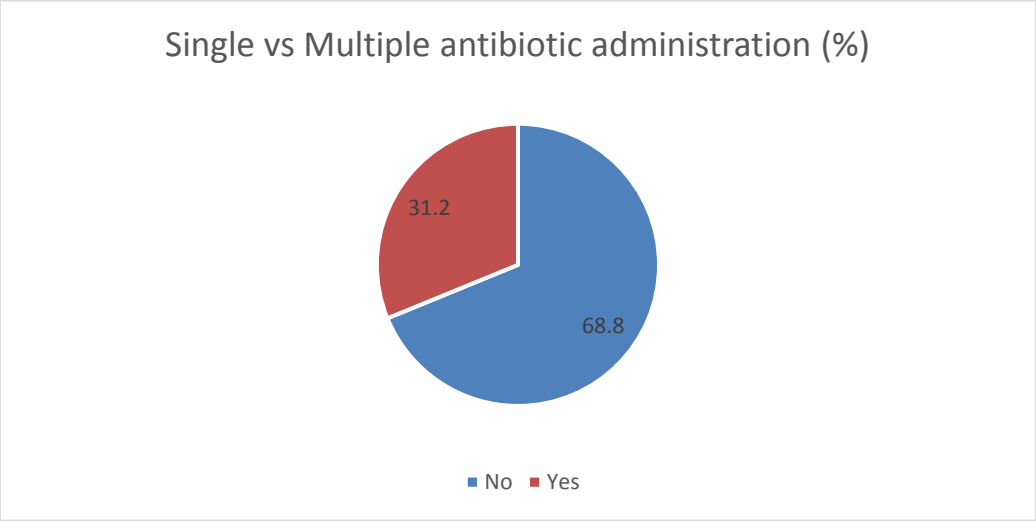

Figure 3-1: Chart representing single vs multiple antibiotic administration in the population

The majority of the cases group (68.8%) was exposed to multiple antibiotic treatments during their stay in the ICU, while occurrence within the control group was much lower (47.7%). This confirms that there is an association between the use of multiple antibiotics and incidence of *Candida* infections.

Patients within the cases group had been exposed to a mean of 5 different antibiotics, while patients within the control group received a mean of 2 different antibiotics during their ICU stay. Further analysis revealed a *p*-value of < 0.001 and an effect size of 0.484, confirming a statistically significant association with moderate effect between exposure to multiple antibiotic agents and the prevalence of *Candida* infections.

The antibiotics administrated throughout a patient's ICU stay represented agents from multiple classes and the incidence of broad-spectrum antibiotic administration was much higher than directed treatment. Cephalosporin and Carbapenem were most prevalent within the cases group. In the worst-case scenario a patient was exposed to ≥14 antibiotic agents during their ICU stay. Refer to figure 3-2.

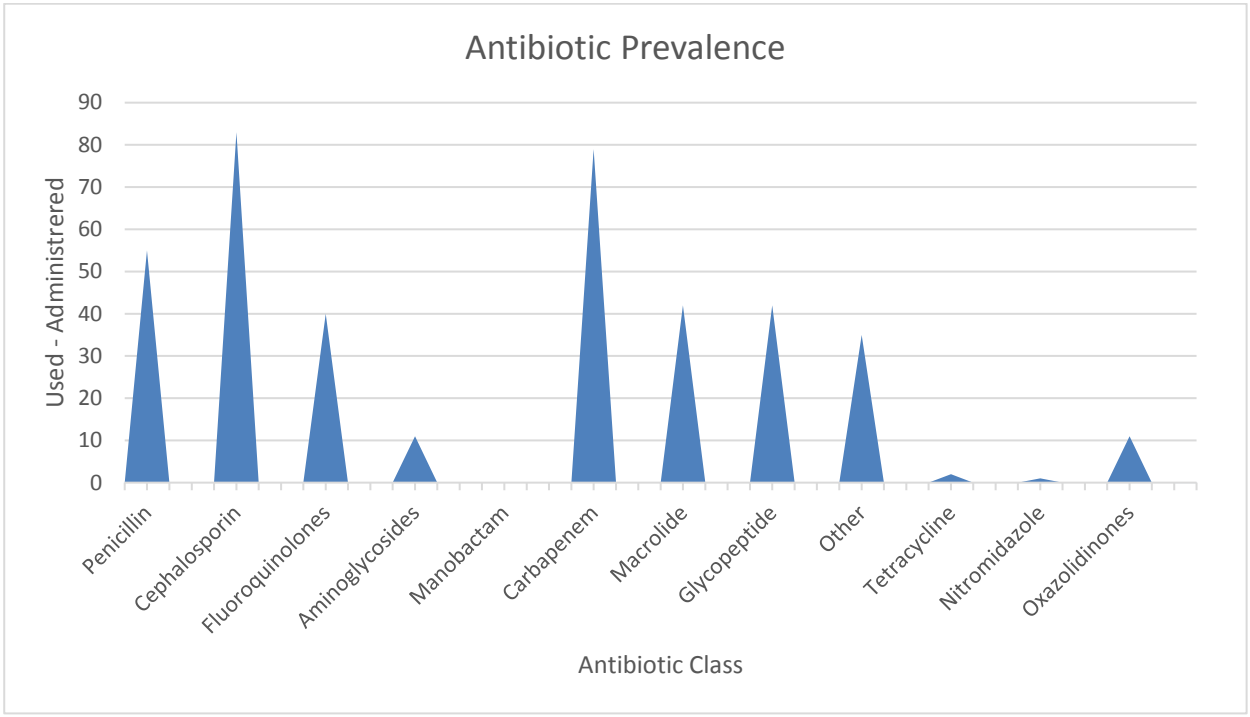

Figure 3-2: Chart representing prevalence of antibiotic administration by class

As the literature and the data analysis now effectively confirmed a linear relationship between the incidence of *Candida* infections and antibiotic exposure, it is important to investigate the effect that the duration of exposure to antibiotics has on the prevalence of these infections.

3.3.1.2 Antibiotic duration

The literature stated that the longer the patient stays in the ICU, the higher the risk of IC becomes (refer to section 2.11). Results related to the duration of antibiotic exposure confirm this and are depicted in Table 3-4.

Table 3-4: Antibiotic duration (All)

Antibiotic duration (All)					
		Frequency	**Percentage**	**Valid percentage**	**Cumulative percentage**
Valid	< 7 days	105	52.0	52.0	52.0
	≥ 7 days	97	48.0	48.0	100.0
	Total	202	100.0	100.0	

Antibiotic exposure duration was divided into two groups: Exposure for ≥ 7 days or exposure for < 7 days (refer to section 1.2). Figure 3-3 provides an explanatory analysis of duration distribution in the population.

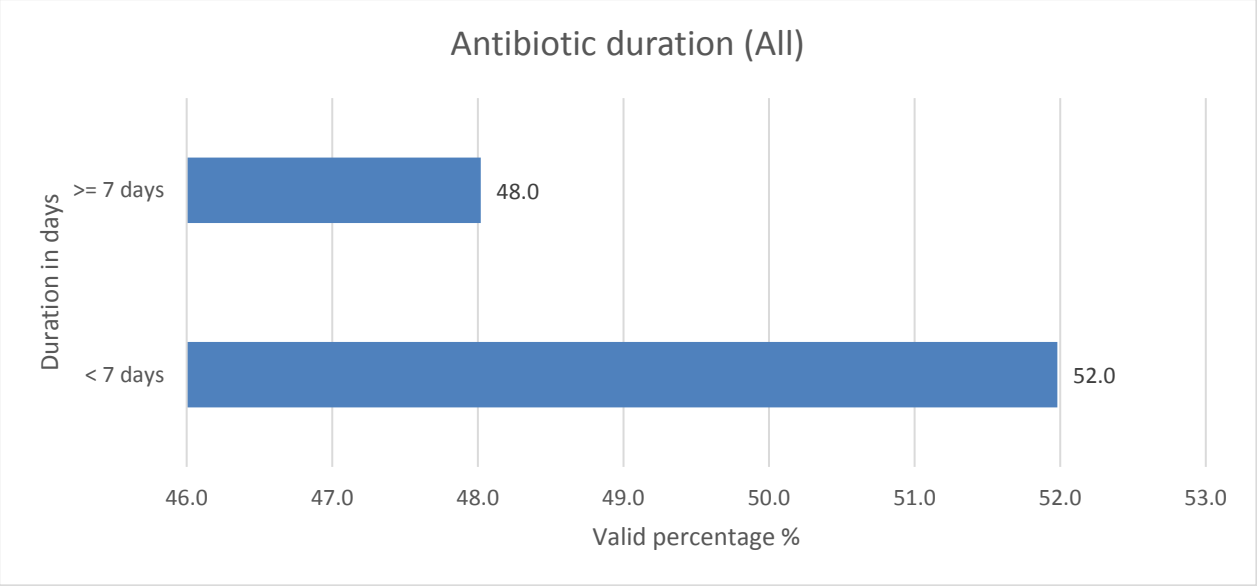

Figure 3-3: Chart representing the antibiotic duration distribution in the population

Duration of antibiotic administration exceeded 7 days in 48% of participants, while 52% of participants received antibiotic treatment for less than 7 days.

Further analysis revealed that 81 patients (85.0%) within the cases group were exposed to multiple antibiotics for a LOS ≥ 7 days. Data analysis in table 3-5 confirm that prevalence of *Candida* infection is notably higher in patients exposed to antibiotic treatment exceeding a LOS of 7 days.

Table 3-5: Crosstab cases group (1) control group (2): antibiotic duration (All)

Crosstab study population (1) control group (2): antibiotic duration (All)					
			Antibiotic duration (All)		Total
			< 7 days	≥7 days	
Cases group (1) control group (2)	Cases group	Count	14	81	95
		% within cases group (1) control group (2)	14.7%	85.3%	100.0%
		% within antibiotic duration (All)	13.3%	83.5%	47.0%
	Control group	Count	91	16	107
		% within cases group (1) control group (2)	85.0%	15.0%	100.0%
		% within antibiotic duration (All)	86.7%	16.5%	53.0%
Total		Count	105	97	202
		% within cases group (1) control group (2)	52.0%	48.0%	100.0%
		% within antibiotic duration (All)	100.0%	100.0%	100.0%

The chi-square test *p*-value was reported as $p < 0.001$, which confirms this correlation as statistically significant (refer to table 3-6) and ultimately confirms that extended duration of antibiotic exposure increases the incidence of secondary *Candida* infections.

Table 3-6: Symmetric measures *antibiotic duration (All)

Symmetric measures			
		Value	**Approximate significance**
Nominal by nominal	Phi	-0.702	0.000
	Cramér's V	0.702	0.000
N of valid cases		202	

Furthermore, the Cramér's *V*-value was reported as < 0.702, confirming that the correlation is practically significant and that the longer a patient is exposed to antibiotic treatment, the higher the prevalence of secondary *Candida* infections.

Since the data confirmed the statistically significant relationship between antibiotic exposure and the prevalence of *Candida* infections, it is important to review if the unauthorised application of MDI or manufacturer indicated duration has any effect on this relationship.

3.3.2 Manufacturer dose indication and duration

Manufacturer dosage indication (MDI) and manufacturer indicated duration for medicine refer to the authorised indication, duration and specific dosage of that drug to treat a particular disease as per development research results (refer to section 2.10).

Table 3-7: Manufacturer dose indication (MDI) vs non-MDI

MID versus Non-MDI					
		Frequency	**Percentage**	**Valid percentage**	**Cumulative percentage**
Valid	MDI	136	67.3	67.3	67.3
	Non-MDI	66	32.7	32.7	100.0
	Total	202	100.0	100.0	

From the data in table 3-7, it was found that 67.3% of participants received the correct MDI, while 32.7% of participants were exposed to non-MDI administrations.

Table 3-8: Manufacturer dose indication (MDI) vs non-MDI

Crosstab cases group (1) control group (2) * MID vs Non-MDI					
			MID vs non-MDI		Total
			MDI	non_MDI	
Cases group (1) Control group (2)	Cases group	Count	45	50	95
		% within cases group (1) control group (2)	47.4%	52.6%	100.0%
		% within MDI vs non-MDI	33.1%	75.8%	47.0%
	Control group	Count	91	16	107
		% within cases group (1) control group (2)	85.0%	15.0%	100.0%
		% within MID vs non-MDI	66.9%	24.2%	53.0%
Total		Count	136	66	202
		% within cases group (1) control group (2)	67.3%	32.7%	100.0%
		% within MID vs non-MDI	100.0%	100.0%	100.0%

Incorrect MDI administration was more prevalent in the cases group than within the control group. In the cases group, only 47.4% of patients received the correct MDI during their stay in the ICU, whereas within the control group, 85% of patients received the accurate MDI.

Analysis within table 3-8 confirms a clear association between incorrect MDI administration and the prevalence of *Candida* infections. The data indicates that application of incorrect MDI increases the risk for *Candida* infection, a relationship confirmed as statically significant ($p < 0.001$) with a moderate effect size (0.401).

3.3.2.1 Manufacturer dose interval

The treatment regimen of ICU patients also includes the manufacturers' dosage interval for each treatment. When there is sufficient supporting evidence to approve a medicine for a specific indication and dosage interval, this specification becomes the approved dosage, indication and dosage interval.

Table 3-9 recorded that the accurate manufacturer indicated dosage interval was administered to 68.3% of participants and a non-manufacturer indicated dosage interval was administered in 31.7% of participants.

Table 3-9: Manufacturer dose interval versus non-manufacturer dose interval

Manufacturer dosage interval (No = 1 Yes=2)					
		Frequency	**Percentage**	**Valid percentage**	**Cumulative percentage**
Valid	No	64	31.7	31.7	31.7
	Yes	138	68.3	68.3	100.0
	Total	202	100.0	100.0	

In the cases group, only 50.5% of patients received the manufacturer indicated dosage interval, while 75.0% of the control group received the manufacturer indicated dosage interval. When the incorrect dosage interval is administered, it affects the performance and side effects of the antibiotic in the patient (refer to section 2.10), predisposing the patient to a fungal infection.

This result supports the literature review as prevalence of *Candida* infections was higher in patients that received the incorrect dosage interval than that of patients receiving the authorised dosage interval. The effect size of the correlation was moderate (0.382).

The result reiterates the importance of following the correct rationale during selection of antibiotic treatment by correctly identifying the infective organism, the site and severity of the infection, and determining the appropriate dose, duration and finally the route of administration (refer to section 2.10.2).

3.3.3 Route of administration

Data related to the route of administration are depicted in Table 3-10.

Table 3-10: Route of administration

Route of administration					
		Frequency	**Percentage**	**Valid percentage**	**Cumulative percentage**
Valid	Oral	2	1.0	1.0	1.0
	IV	200	99.0	99.0	100.0
	Total	202	100.0	100.0	

The majority of participants (99%) received IV antibiotic treatment, while only one participant received oral treatment. This result is a logical approach given the nature of the ICU environment and the condition of patients within this ward. The correlation between the route of administration and prevalence of *Candida* infections was found to be weak.

3.4 Demographic data

The results and discussions of the empirical investigation successfully determined the prevalence of secondary *Candida* infections in the specific ICU and also confirmed antibiotic exposure as a direct risk factor for secondary *Candida* infections. As final analysis, specific host-related demographic attributes were assessed for relevance.

3.4.1 Demographic data: Age

The literature review identified specific demographic attributes (age and sex) as host-associated risk factors for *Candida* infections (refer to section 2.6). This prompted further investigation of these specific attributes within this population and is noted in figure 3-4.

The highest percentage of participants within the cases group, 70.53%, was represented by the age group 65 years and older. This is visually illustrated in figure 3-4.

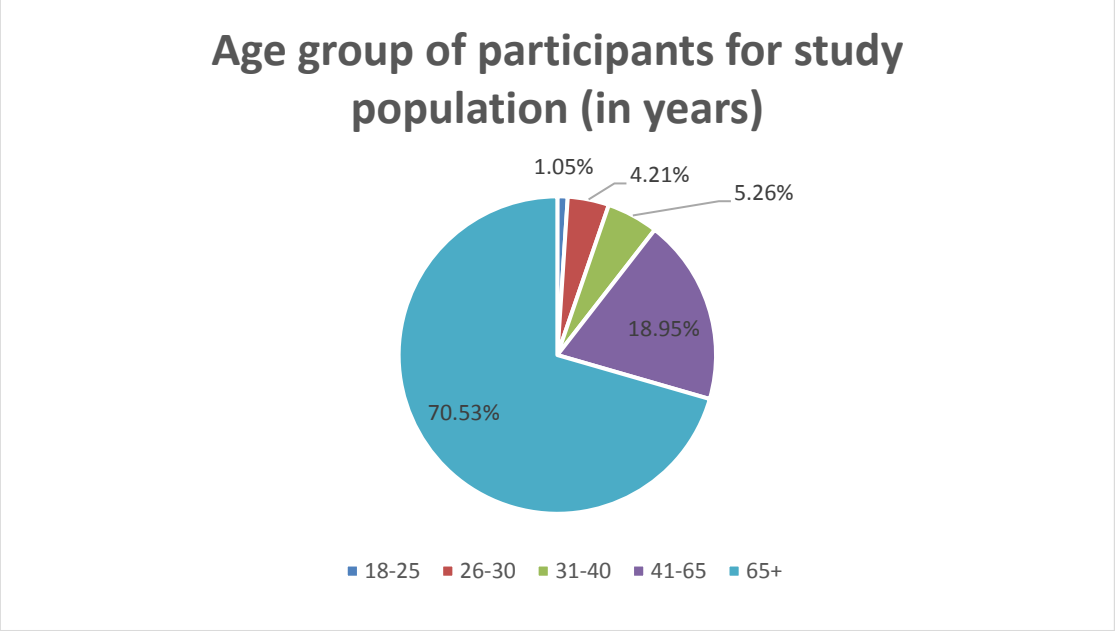

Figure 3-4: Chart representing the age group of participants for the population

A large portion of the control group (31.78%) was aged between 41 and 65 years. A slightly larger part of the group (54.21%) represented the age group 65 years and older. The age distribution of the control group is described in detail in figure 3-5.

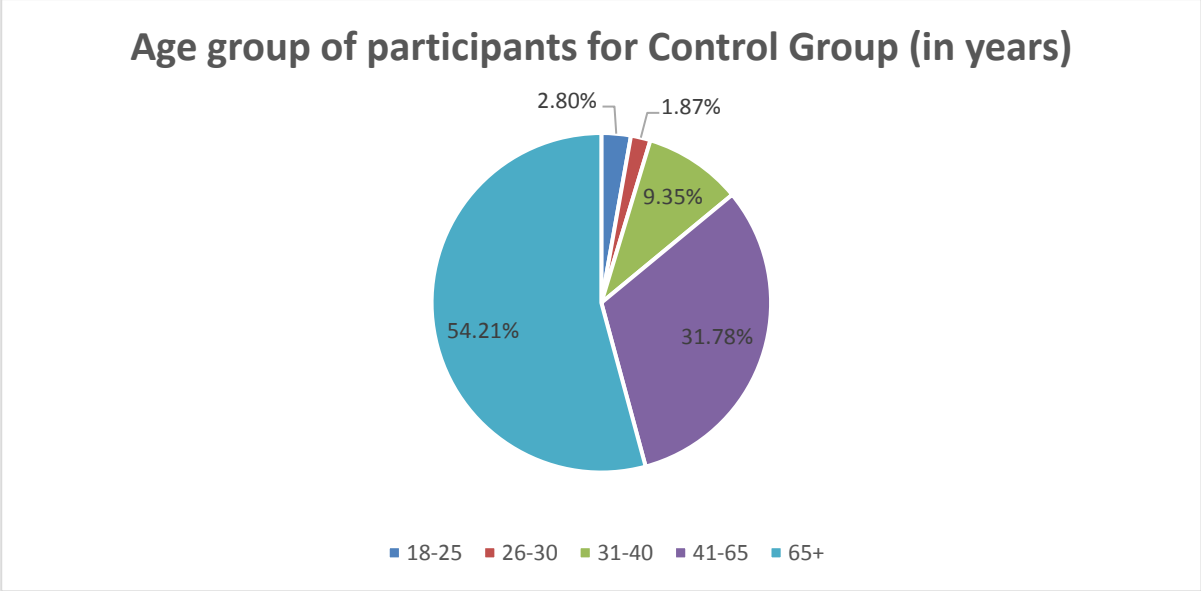

Figure 3-5: Chart representing the age group of participants for the control group

The literature and data analysis confirmed age a leading host-related risk for *Candida* infection. The data needed to be analysed with reasonable attention to get a clear picture regarding the impact of age on the prevalence of *Candida* infections within this specific population, and therefore, descriptive statistics were utilised as depicted in tables 3-11 and 3-12.

Table 3-11: Descriptive analysis: Demographic age

Descriptives				
			Statistic	**Standard error**
Age	Mean		64.39	1.199
	95% confidence interval for mean	Lower bound	62.03	
		Upper bound	66.76	
	5% trimmed mean		65.19	
	Median		67.00	
	Variance		290.598	
	Standard deviation		17.047	
	Minimum		19	
	Maximum		93	
	Range		74	
	Interquartile range		22	
	Skewness		-0.782	0.171
	Kurtosis		-0.035	0.341

The data clearly shows that *Candida* infections are more prevalent in elderly patients, with the largest portion of the cases group consisting of patients 65 and older, while a more equal age distribution is seen within the control group between adult and geriatric patients. Percentiles were used to measure the value below which gives the percentage of occurrence in the respective groups to allow for comparison between the cases and control groups.

Table 3-12: Percentiles: Demographic age

Percentiles								
		Percentiles						
		5	**10**	**25**	**50**	**75**	**90**	**95**
Weighted average (definition 1)	Age	30.15	37.00	56.00	67.00	78.00	82.70	87.00
Tukey's Hinges	Age			56.00	67.00	78.00		

The youngest patient was aged 19 years, while the oldest patient was aged 93 years. Within the cases group 70.53% was geriatric patients while only 54.2% of the control group was elderly. A median age of 67 years was noted for the cases group which supports the literature review. The

data analysis confirms that prevalence of fungal infections is much higher in geriatric patients than other age groups (refer to section figure 3-6).

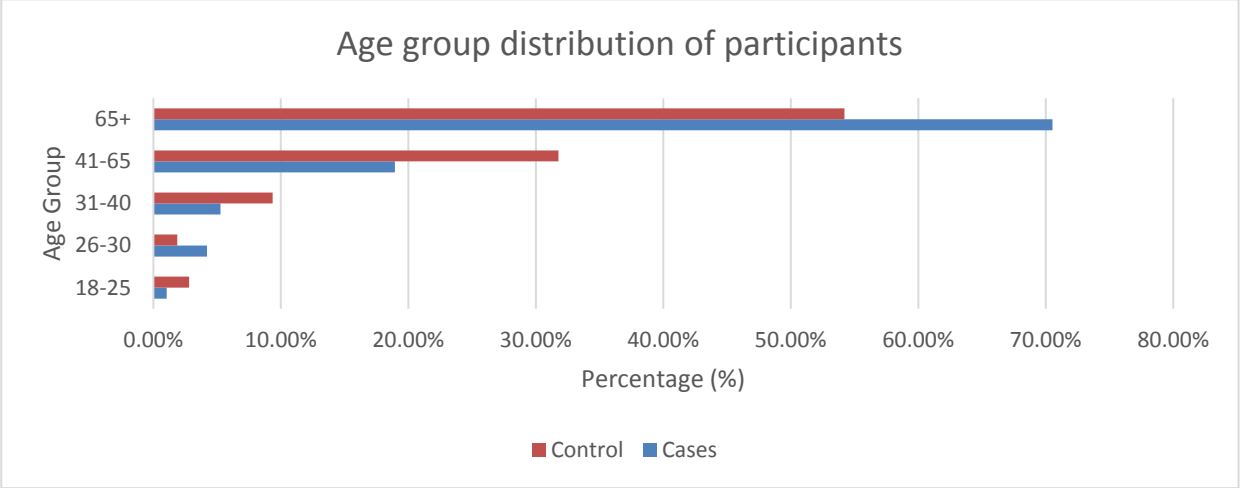

Figure 3-6: Chart representing the age group distribution of participants

The data confirms a significant correlation between age and prevalence of secondary *Candida* infections within the population and specifically confirms an increased prevalence of fungal infections in geriatric patients within this ICU setting. The volume of senior patients that required the services and support provided by this specific ICU also underscores the literature as reality (refer to section 2.6).

3.4.2 Demographic data: Sex

The literature identified gender-related differences regarding prevalence of fungal isolates, with female subjects showing a higher number of fungal isolates than males (refer to section 2.3).

Table 3-13: Gender distribution of participants

Gender					
		Frequency	**Percentage**	**Valid percentage**	**Cumulative percentage**
Valid	Male	97	48.0	48.0	48.0
	Female	105	52.0	52.0	100.0
	Total	202	100.0	100.0	

As seen in table 3-13, the population showed a slightly higher incidence of fungal infection in the female population than the male population, which supports the differences noted during the literature review, albeit only slightly.

Male participants represented 48% of the population, while female participants represented 52% of the population. Ultimately the review of the demographic analysis provided a picture which supported the literature review and allowed for the conclusion that both age and sex does impact the prevalence of *Candida* infections within the ICU.

3.5 Conclusion statement

This chapter outlined data analysis for the study. The demographic data were summarised and illustrated in graphs; Pearson's correlation analysis was done between all the constructs; and the Cohen's effect size (d-value) comparisons were summarised and interpreted.

In summary, the data confirmed significant correlations which support that antibiotic exposure is a risk factor for secondary *Candida* infections in the ICU. In Chapter 4, the conclusions, limitations and recommendations of the research will be discussed.

CHAPTER 4: CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

4.1 Introduction

This chapter contains the conclusions and a summary of the results attained in this study. It therefore concludes this inquiry. Several research objectives were posed during the commencement of this study. The conclusion, results and recommendations thereof are presented below. The limitations of the study are also noted in this chapter.

4.2 Conclusions

The conclusions of this study are framed according to the specific research aims.

4.2.1 Literature aims

The following three literature aims were set for this study in Chapter 1. Conclusions from this study are included.

4.2.1.1 Define *Candida* infections and review the literature for the epidemiology, risk factors, pathogenesis, clinical presentation, diagnosis and treatment of these infections

The epidemiology, risk factors, pathogenesis, clinical presentation, diagnosis and treatment of *Candida* infections are discussed in detail in Chapter 2.

- Epidemiology

As seen in section 2.4, it can be concluded that ICU patients are especially at risk for opportunistic fungal infections. From the literature review, it was confirmed that the incidence of IC has increased in ICUs throughout the world. A similar increase has also been noted in several population-based studies, which indicated that the incidence of IC varies from one country to another.

The literature also confirmed that *C. albicans* is still considered the main causative species of candidaemia worldwide, although other, non-*albicans* spp. are also increasingly responsible for IC. The epidemiological review indicates that all *Candida* spp. represents a serious challenge to the healthcare system and a notable threat to ICU patients specifically.

- Risk factors

Intensive care patients are at high risk of fungal infections. The identification of risk factors, such as systemic antibacterial use, total parenteral nutrition, abdominal surgery, age, extended ICU stay and colonisation, enables clinicians to successfully identify patients with a high risk of invasive candidiasis.

In answer to the research question, the literature verified that the following specific risk factors predispose ICU patients to secondary *Candida* infections: colonisation, a lengthy LOS in the ICU and exposure to antibiotic treatments (Caggiano *et al.,* 2011:7041; Eggimann & Pittet, 2014). This review supports the data and confirms that a prolonged LOS increases the prevalence of *Candida* infection (Pfaller *et al*., 2011:120-127; Yapar, 2014:102).

The combination of a long LOS and the abundant exposure to antibiotics especially affects ICU patients and results in colonisation, and ultimately *Candida* infections (refer to section 2.6), confirming that the longer the patient stays in the ICU, the higher the risk for IC (Pinhati *et al*., 2016:433).

- Pathogenesis

As seen in section 2.5, it can be concluded that the literature review provided a clear overview of the importance of *Candida* as a public health issue; *C. albicans* is still considered the most common human fungal pathogen worldwide (Marins *et al,* 2018:187). *Candida* infections are still accepted as the most common opportunistic nosocomial fungal infections in surgical and medical ICU patients.

The literature confirmed that *Candida* is normally a harmless commensal organism; however, as an opportunistic pathogen, it results in severe, life-threatening infections affecting vulnerable patients.

Candidaemia as a life-threatening condition is associated with considerable morbidity and with the increase in the number of individuals who are sensitive to invasive fungal infections, fungi are reported more frequently as pathogens.

- Clinical presentation

Candida infections may present in three subgroups: superficial *Candida* infections, IC or candidaemia. Clinical signs of severe *Candida* infection manifest early, but lack specificity until late in the course of the disease, which is a particular challenge for diagnosis and appropriate treatment.

Invasive candidiasis may involve all tissue and organs and so it may present with virtually all clinical signs and symptoms inherent to the ICU, making diagnosis extremely difficult. This increases the risk for morbidity, high mortality, and the significant use of additional resources (refer to section 2.7).

- Diagnosis

As seen in section 2.8, it can be concluded that the diagnosis of invasive candidiasis is made most easily in those patients with positive blood cultures, but the low sensitivity of blood cultures means that some patients with deep-seated infection may be missed and often the diagnosis of IC is only made late during an infection or during an autopsy.

- Approach to antifungal therapy

The literature review confirmed that multiple strategies are used in the treatment of *Candida* infections in the ICU (refer to section 2.3).

The different approaches are selected based on the best approach related to the given clinical situation. The use of anti-fungal treatment for candidiasis in ICUs usually falls into one of four categories:

Prophylaxis should be restricted to very specific subgroups of patients in whom it has been demonstrated to be useful, whereas pre-emptive therapy should be used in colonised patients or with high-risk scores. Empirical therapy should only be applied in septic patients who are not responding to appropriate antibacterial treatment. Finally, definitive treatment is applied in patients reflecting a positive diagnosis based on blood or tissue culture results. It is species specific and targeted treatment.

In summary, the rapid diagnosis as well as early initiation of the appropriate antifungal therapy reduces morbidity and mortality in ICU patients.

Antifungal treatment is divided into three main groups currently available, and these include: polyenes, azoles and echinocandins. Since physicians retain the authority to prescribe antifungal treatment as prophylaxis, empiric therapy or preventive treatment while waiting for the diagnosis to be confirmed, there is a definite degree of excessive exposure to antifungal until definitive therapy can be applied.

4.2.1.2 Review the literature for antibiotic uses, applications and side-effects with specific focus on risks associated for developing secondary *Candida* infections due to antibiotic exposure

Antibacterial therapy provides one of the only pharmacologic treatments that cures disease. The structural and metabolic differences between bacteria and human cells enable the antibiotics to cause selective toxicity to the bacterial organisms without causing any damage to the host cells. Antibiotics are applied to treat infections and can be either bactericidal or bacteriostatic. Antibiotics work by exploiting the differences that exist in cell structures between bacterial cells and animal cells.

Currently, there are a number of classes of antibiotics/antibacterial agents that are commonly used in clinical practice to treat bacterial infections, as described in detail in Annexure G. These different antibiotics have various target sites on the bacteria, which include the cell wall, cell membranes, ribosomes, nucleic acids, bacterial cellular metabolism and bacterial cellular enzymes (refer to section 2.10.2).

The different antibiotics also have many different mechanisms by which they inhibit the proliferation, and actively destroy bacteria. Antibiotic working mechanisms include inhibition of cell wall synthesis, disruption of cell-membrane function, inhibition of protein synthesis (both 50S and 30S) 4), inhibition of nucleic acid synthesis (both the DNA synthesis and RNA synthesis) and action as antimetabolites as described in Annexure G.

Antibiotics are probably the most successful family of drugs so far developed for improving human health. Antibiotics have been used very efficiently to prevent and treat infections globally and from the literature it was confirmed that antibiotics are still one of the most commonly used agents in ICUs globally.

Although antibiotics have been extremely successful in the treatment of illness, the total amount of antibiotics used is considered as a critical factor for the emergence and dissemination of antibiotic resistance. The literature review indicated that the prevalence of resistance is increasing. The increases in antibiotic resistance correlate with the increase in antibiotic consumption and this has a negative impact on human health (refer to section 2.10.3).

Accurate diagnosis of diseases and the appropriate application of antimicrobials are essential for successful clinical outcomes. As per the literature review in Chapter 2, it is clear that appropriate application of antibiotics adds benefits to the patient, the healthcare system and society.

From the literature review, it is clear that the administration of antibiotics significantly influences the function of the defence mechanisms of the human body, the immune system and ultimately the body's ability to resist infection against opportunistic infections (Alfouzan *et al.,* 2015:24). Critically ill patients without any other risk-factors apart from a prolonged exposure to antibiotics generally develop complex immunological derangements, which put them at risk for developing IC.

The literature review confirmed in Chapter 2 section 2.10 that the prevalence of fungal infections is associated with antibiotic therapy because antibiotics enhance the invasiveness of the fungi, depressing the intestinal flora and affecting the ecology of the GIT systems in profound ways, resulting in lasting changes to the GIT, which results in the overgrowth of fungi (Marins *et al,* 2018:187).

The direct effect of administered antibiotics is the removal of organisms that protect the host from fungal infections predisposing the host to infection resulting in flourishing *Candida*. Due to the selective pressure on the digestive system caused by antibiotics, modification of the intestinal flora occurs, which inhibits commensal organisms and allows for the overproduction of fungi, which results in colonisation and infection.

It appears that the major predisposing factors for colonisation consist of prolonged use of antibiotics and the use of multiple antibiotic classes simultaneously, subsequently resulting in a higher prevalence of IC (Jacobs *et al.*, 2015:92; Yapar, 2014:102).

4.2.1.3 Define and clarify the relationship between length of stay, antibiotic exposure and secondary *Candida* infections

The literature review provided evidence that antibacterial agents can increase the likelihood of *Candida* spp.-related infections (Caggiano *et al.,* 2011:7041; Eggimann & Pittet, 2014). The mechanisms by which the antibiotics increase the hazard of candidiasis entail more than simple overgrowth by *Candida* in the absence of competing organisms.

Tissue damage by the antibiotic as well as overgrowth and endotoxin released by the proliferating *Candida* spp. convert the *Candida* to an invasive form. The invasion could be mediated by the local damage to the GIT caused directly by the antibiotics, or indirectly by the endotoxin released from the proliferating *Candida* spp.

The direct depression of host-defence mechanisms is also a serious contributor to the already depressed immunological mechanisms of an ICU patient. In the ICU patients, the additional

compromise of the host defences, or an increase in the population of the potential pathogen, is sufficient to allow for its spread throughout the body, causing IC.

The literature confirmed that there is a direct association between secondary *Candida* infections and prolonged antibiotic exposure in the ICU (Noni *et al.,* 2015:419). The literature review and results suggest that a patient exposed to antibiotic treatment for a longer period of time in the ICU is more likely to develop secondary *Candida* infections. The relationship between antibiotic exposure with a prolonged LOS and colonisation is linear, meaning that prolonged exposure increases the risk of IC in ICU patients.

4.2.2 Empirical investigation objectives

The empirical investigation objectives included determination of the prevalence of secondary *Candida* infection in the specific ICU for the specified study period as well as investigation of antibiotic exposure as a risk factor for secondary *Candida* infections. These objectives will be described in the sections below.

4.2.2.1 Study population distribution & demographics

The cases- and control groups were equally represented which ensured comparable results (refer to Table 3-2). Age was confirmed as a risk factor for an extended LOS and also an attributable risk factor for candidaemia. The data analysis confirmed the correlation between age and prevalence of secondary *Candida* infection within the study population and specifically confirmed an increased prevalence of fungal infections in geriatric patients within this ICU setting.

The global trend of increased prevalence of older patients admitted in the ICU globally (refer to section 2.6), was also confirmed within this specific setting. The data supported the literature as it was evident in the results that the prevalence of fungal infections was much higher in the elderly, with smaller portions of the population aged younger than 65 years.

A median age of 67 years was noted for the study population and supports claims that prevalence of fungal infections is much higher in senior patients (refer to section 2.6). The research confirmed that age is a specific risk factor for candidaemia and that elderly patients are more susceptible to this disease within the ICU.

4.2.2.2 The literature indicated that *Candida* spp. are more frequently found as commensal organisms in females. The population level analysis revealed gender-related differences, with female subjects showing a higher prevalence for *Candida* infections than male subjects, which supports the results noted in the

literature review (refer to section 2.3), however this was only slightly significant for the specific population. Antibiotic use within the study population

Broad-spectrum antibiotic agents were most prevalent within the study population and high prevalence of simultaneous administration of multiple antibiotic agents were evident. The antibiotics administrated represented agents from multiple classes and were administered concurrently. There was a higher prevalence of broad-spectrum antibiotic administration than directed treatment (refer to section 3.2.8).

As seen from the data analysis in section 3.2.13, a predominant trend for concurrent use of multiple antibiotics within the population as well as increased incidence of *Candida* infections in patients exposed to antibiotics was confirmed and was statistically significant.

4.2.2.3 Antibiotic duration

The data confirmed higher prevalence of *Candida* infections in patients exposed to antibiotic treatment for periods longer than seven days. The results support the literature review and confirmed that prevalence of *Candida* infections is much higher in patients with an extended LOS (refer to section 3.2.6).

4.2.2.4 Manufacturer dose indication and duration

The results confirmed that *Candida* infections are more prevalent in patients receiving the incorrect administration of dosage indication or interval than patients that receive the appropriate treatment. This result drives the need for a standardised and monitored approach to antibiotic administration aligned with the appropriate manufacturer dose indication and duration.

4.2.2.5 Route of administration

The study only found a weak correlation between route of administration and prevalence of *Candida* infection within the population. It was however evident that IV was the preferred method of administration within the setting and as oral treatment was limited, further investigation of route of administration as risk for *Candida* infection would be valuable.

4.2.2.6 Empirical investigation summary

The study successfully achieved the research objectives as noticeable correlations between antibiotic exposure and IC, as well as correlations between the prevalence of *Candida* infection and the length of exposure was confirmed by the data analysis in Chapter 3:

The following practical significant associations were confirmed:

- Age - Elderly patients were proven to be more susceptible to IC than younger patients within the same population (refer to section 3.2.3)

- Multiple antibiotic administration is a direct risk for prevalence of *Candida* infections. There is a strong practical significance between duration of antibiotic exposure (exceeding 7 days) and prevalence of secondary *Candida* infections (refer to section 3.2.8)

- Application of non-MDI has a direct impact on prevalence of secondary *Candida* infections. Application of non- MDI was confirmed as a risk for prevalence of secondary *Candida* infections in the specific ICU (refer to section 3.2.16).

Ultimately the empirical investigation confirmed antibiotic use as a direct risk for *Candida* infection in ICU patients of this specific ICU.

4.2.2.7 Determine the prevalence of secondary *Candida* infection in the specific ICU for the specified study period

The prevalence of secondary *Candida* infection in the specific ICU for the specified study period was determined. A total of 202 valid cases were included in the study; 95 patients presented with a *Candida* infection unrelated to their original diagnosis. This represented 47% of the population in the specific ICU during the specified time period.

Almost half of the population contracted a fungal infection during their stay within the ICU. The effect size confirmed a strong association between admission to the ICU and prevalence of secondary *Candida* infections with an effect size of 0.702.

The statistical analysis successfully determined the prevalence of secondary *Candida* infection within the specific ICU for the specified study period.

4.2.2.8 Investigate antibiotic exposure as a risk factor for secondary *Candida* infections

From the above summary and the statistical analysis, the results indicate that there is a significant association between the prevalence of secondary *Candida* infections and exposure to multiple antibiotics for an extended period of time.

There is a meaningful statistical association between antibiotic exposure duration and the group (study or control) classification. The effect size is classified as large: effect size = 0.702. Therefore, a practical meaningful association between group (study/control) and antibiotic duration is proven. The p-value is smaller than 0.05.

A clear significant relationship also exists between deviation from the MID as well as manufacturer dosage interval resulting in increased prevalence of secondary *Candida* infections with a p-value reported as < 0.001.

The data confirmed a pattern with meaningful associations identified between the duration of antibiotic exposure, MDI vs non-MDI and application of multiple antibiotic treatments all resulting in the prevalence of secondary *Candida* infections.

The researcher confirmed that there is a direct association between secondary *Candida* infections and prolonged antibiotic exposure in the specific ICU from a detailed literature review. The researcher also confirmed meaningful associations between secondary *Candida* infections and antibiotic exposure, proving that there is a statistical association between the two concepts.

4.3 Recommendations

Recommendations for healthcare practice, development, policy and future research will be discussed below.

4.3.1 Recommendations for healthcare institutions

The high prevalence of secondary *Candida* infections in this ICU constitute a significant problem. The root cause of this problem is multifactorial, and the core issues are clear. The prevalence of *Candida* infection correlates directly with selective pressure resulting from inappropriate use of antibiotic application, dosage and duration.

The study confirmed the prevalence of *Candida* infection within this ICU as statistically significant and since antibiotic exposure was confirmed as a major risk for incidence of IC, it is important to enable the staff to collect and share sound scientific data and practices essential to patient care to reduce risk.

Antibiotic therapy for ICU patients should always include the accurate administration, MDI and duration of agents to avoid inappropriate treatment, overuse or abuse. Appropriate antibiotic stewardship in ICUs should include rapid identification and optimal treatment of bacterial infections, based on pharmacokinetic-pharmacodynamic characteristics, thereby avoiding unnecessary administration of antibiotics, shortening the duration of exposure, and reduction in the number of patients receiving incorrect antibiotic therapy.

Implementation of multidisciplinary teams will improve the quality and efficacy of patient care by including experts that is able to rapidly identify infections and apply the accurate empirical therapy.

This strategy requires appropriate selection, application and management of antibiotic treatment guided by the expertise of a multidisciplinary team.

Accurate MDI administrations, as well as accurate manufacturers indicated dosage intervals, should be evaluated and encouraged with protocols evaluated by a clinical pharmacist in the ICU. Staying informed about developments in ICU best-practice would be extremely beneficial for the well-being of ICU patients and responsible antibiotic administration would decrease the incidence of *Candida* infections in the ICU.

Treatment should be driven by proven results and aim to focus on accurate monotherapy whenever possible. Shortening treatment to less than 7 days for patients must be applied, based on the clinical response and supported by microbiological results.

Since dosage and duration specifically impacted the results, the knowledge and experience of a clinical pharmacist would be extremely valuable for appropriate assessment, administration and monitoring within ICU patients.

Antibiotic administration as risk factor for the development for secondary *Candida* infections should be emphasised in curriculums of medical and nursing schools, as it is essential that healthcare professionals are aware of the effect that they could have on vulnerable patients in the ICU.

Antibiotic stewardship programmes and education of practising healthcare professionals in workshops should be encouraged to ensure proactive monitoring and prevention of risks associated with secondary *Candida* infections.

4.3.2 Recommendations for future research

Valuable and descriptive results were reported in this small study population, but it is recommended that the findings be verified in larger study populations in various institutions in South Africa, as these will help to understand fully all aspects involved, better focus on the best way to confirm results reported, and help generalise the outcomes for the outcomes of this study.

This study may form a platform for future studies to further expand on with the following recommendations that can be made:

4.4 Limitations of the study

The study was focused on the fact that *Candida* is indigenous to all patients and excludes the possibility that secondary infections are caused by external transfer or contamination, which might also be a factor to include in future studies.

There was room for error in diagnosis in terms of primary or secondary infections. Not all patients were tested for *Candida* spp. infections at admission or during their ICU stay if there were no physical signs of a *Candida* infection. As this study was a retrospective descriptive investigation, the researchers did not have any control over the availability and correctness of the collected data. Some data on critical time-points could not be recorded and contributed to incomplete patient profiles.

The study was limited to only one hospital and one ICU and this limited the number of participants and the accuracy of the findings with conclusions drawn being specifically relevant to this study. Please refer to section 1.10.10 for supporting arguments that mitigate this limitation and indicate that it does not affect the validity and generalisability of the study.

The study was limited to investigation of antibiotics in general, and attention to specific classes of drugs could be expanded in future research to examine the effect of specific drugs on the prevalence of *Candida* infections.

This study was completed using a convenience sample in one organisation. As a result, the generalisability of the results can be interrogated. The researcher notes that generalisation is not the aim of all research. Similar research is aimed primarily at groundwork studies.

4.5 Strengths of the study

As seen from the literature, it is preferable that a modest descriptive study does include many different groups of people within a study, as homogeneity is ensured when groups with similar ways of life are included within the same cause.

By limiting the study to one location, it enabled the researcher to define the relationship between the administration of antibiotics and secondary *Candida* infection within the population. By restricting the catchment area, the researcher was able to get clear results for the specific setting.

Reliable data were described with validity and reliability, the demographic data were summarised and illustrated in pie graphs, Pearson’s correlation analyses were done between all the constructs and the Cohen’s effect size (d-value) comparisons were summarised and interpreted as well as

the means and standard deviations. These all assisted in eventually answering the research questions and supporting the study objectives.

The retrospective design structure enabled the researcher to answer the defined research questions utilising the validated data, thereby ensuring validated results.

A major strength for this cohort study was the possibility to study multiple exposures and multiple outcomes in one cohort. Even rare exposure could be studied; the combined effect of multiple exposures on disease risk could be determined and this enabled the researcher to reach the research objectives.

4.6 Summary

In this chapter, the limitations of the study, recommendations for future endeavours and research were evaluated. The findings of the study revealed moderate and strong associations between the prevalence of secondary *Candida* infections and exposure to antibiotic treatment with regard to evidence from literature and the empirical results collected from the specific population. In conclusion, the study may provide valuable insight that may contribute to prevention of the under-recognised effect of antibiotic exposure to vulnerable ICU patients, emphasising the need for future research in terms of effective management strategies of comorbidities that can reduce the burden of illness in ICU patients.

4.7 Reflection of the study

The results indicated a clear and direct correlation between exposure to antibiotics and prevalence of secondary *Candida* infections in ICU patients. Antibiotics are one of the most frequently used drugs worldwide and from the results it is clear that patients are getting sicker due to the rampant usage of antibiotics resulting in secondary nosocomial infections. Patients are often treated with antibiotics without cause putting them at risk for development of secondary *Candida* infections.

All healthcare professionals must assume responsibility for the appropriate and responsible treatment of patients and, as the custodians of medicine, pharmacists should especially provide guidance and support for accurate treatment regimens within the healthcare team.

By applying antibiotics in a responsible manner and managing patient care as a multidisciplinary team, patients will benefit, medical personnel will enhance patient outcomes and the overall risk for secondary *Candida* infections will be reduced.

REFERENCE LIST

Akbari, F. & Kjellerup, B. 2015. Elimination of bloodstream infections associated with *Candida albicans* biofilm in intravascular catheters. *Pathogens*, 4(3):457-469. DOI: 10.3390/pathogens4030457

Alanis, A.J. 2005. Resistance to antibiotics: are we in the port-antibiotic era? *Archives of medical research*, 36(6):697-705. DOI: 10.1016/j.arcmed.2005.06.009

Alfouzan, W., Dhar, R., Ashkanani, H., Gupta, M., Rachel, C. & Khan, Z.U. 2015. Species spectrum and antifungal susceptibility profile of vaginal isolates of *Candida* in Kuwait. *Journal of medical mycology,* 25(1):23-28. DOI: 10.1016/j.mycmed.2014.10.021

Allegranzi, B., Aiken, A.M., Kubilay, N.Z., Nthumba, P., Barasa, J., Okumu, G., Mugarura, R., Elobu, A., Jombwe, J., Maimbo, M., Musowoya, J., Gayet-Ageron, A. & Berenholtz, S.M. 2018. A multimodal infection control and patient safety intervention to reduce surgical site infections in Africa: a multicentre, before-after, cohort study. *The Lancet infectious diseases*, 17(18):507-515. DOI: 10.1016/S1473-3099(18)30107-5

Aminzadeh, F. & Dalziel, W.B. 2002. Older adults in the emergency department: A systematic review of patterns of use, adverse outcomes and effectiveness of interventions. *Annals of emergency medicine,* 39(3):238-247.

Andersson, D.I. & Hughes, D. 2011. Persistence of antibiotic resistance in bacterial populations. *FEMS microbiology*, 35(5):901-911. DOI: 10.1111/j.1574-6976.2011.00289.x

Antunes, L.M., Ferreira, R.R., Buckner, M.C. & Finlay, B.B. 2010. Quorum sensing in bacterial virulence. *Microbiology*, 156(8):2271-2282. DOI: 10.1099/mic.0.038794-0

Ashley, E.S., Lewis, R., Lewis, J.S. Martin, C. & Andes, M. 2010. Pharmacology of Systemic Antifungal Agents. *Clinical infectious diseases*, 43(1):28-39.

Babbie, E. 2010. The practice of social research. 2nd ed. Belmont, CA: Wadsworth/Cengage.

Badiee, P. & Hashemizadeh, Z. 2014. Opportunistic invasive fungal infections: diagnosis & clinical management. *Indian journal of medical research,* 139(2):195-204. PMC4001330

Bailly, S., Maubon, D., Fournier, P., Pelloux, H., Schwebel, C., Chapuis, C., Foroni, L., Cornet, M. & Timsit, J.F. 2016. Impact of antifungal prescription on relative distribution and susceptibility of *Candida* spp.: trends over 10 years. *Journal of infection,* 72(1):103-111. DOI: 10.1016/j.jinf.2015.09.041

Bamford, C.V., d'Mello, A., Nobbs, A.H., Dutton, L.C., Vickerman, M.M. & Jenkinson, H.F. 2009. *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. *Infection & immunity*, 77(9):3696-3704. DOI: 10.1128/IAI.00438-09

Banerjee, A. & Chaudhury, S. 2010. Statistics without tears: populations and samples. *Industrial psychiatry journal,* 19(1):60-65. PMC3105563

Bassetti, M., Mikulska, M. & Viscoli, C. 2010. Bench-to-bedside review: therapeutic management of invasive candidiasis in the intensive care unit. *Critical care,* 14(6):244. DOI: 10.1186/cc9239

Bbosa, G.S., Mwebaza, N., Odda, J., Kyegombe, D.B. & Ntale, M. 2014. Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance. *Health,* 6(5):410-425. DOI: 10.4236/health.2014.65059

Bersani, I., Piersigilli, F., Goffredo, B.M., Santisi, A., Cairoli, S., Ronchetti, M. P. & Auriti, C. 2019. Antifungal drugs for invasive Candida infections (ICI) in neonates: Future perspectives. *Front paediatrics,* 7:375.

Bessey, P.Q. 2007. Wound care. (*In* Herndon, D.N., *ed.* Total burn care. 3rd ed. Philadelphia, PA: Elsevier. p. 127-135). http://www.amazon.com/Total-Burn-Care-David-Herndon/dp/1416032746 Date of access: 22 Feb. 2017.

Beveridge, T.J. 1999. Structures of gram-negative cell walls and their derived membrane vesicles. *Journal of bacteriology,* 181(161):4725-4733.

Bible. 1989. Life application Bible: King James version. Wheaton, IL: Tyndale.

Bloos, F., Held, J., Schlattmann, P., Brillinger, N., Kurzai, O., Cornely, O.A. & Thomas-Rüddel, D. 2018. (1,3)-β-D-glucan-based diagnosis of invasive Candida infection versus culture-based diagnosis in patients with sepsis and with an increased risk of invasive Candida infection (CandiSep): study protocol for a randomized controlled trial. *Trails,* 19:472. DOI: 10/1186/s13063-018-2868-0

Blumberg, H.M., Jarvis, W.R. & Soucie, J.M. 2001. Risk factors for candida bloodstream infections in surgical intensive care unit patients: the NEMIS prospective multi-centre study: The national epidemiology of mycosis survey. *Clinical infectious disease*, 33(2):177-186. DOI: 10.1086/321811

Boon, C., Deng, Y., Wang, L.H., He, Y., Xu, J.L., Fan, Y., Pan, S.Q. & Zhang, L.H. 2008. A novel DSF-like signal from Burkholderia cenocepacia interferes with Candida albicans morphological transition. *International society for microbial ecology journal*, 2(1):27-36. DOI:10.1038/ismej.2007.76

Brink, H., Van der Walt, C. & Van Rensburg, G. 2012. Fundamentals of research methodology for health care professionals. 3rd ed. Cape Town: Juta.

Burns, N. & Grove, S.K. 2005. The practice of nursing research: conduct, critique and utilisation. 5th ed. St. Louis, MO: Elsevier Saunders.

Caggiano, G., Puntillo, F., Coretti, C., Giglio, M., Alicino, I., Manca, F., Bruno, F. & Montagna, M.T. 2011. Candida colonization index in patients admitted to an ICU. *International journal of molecular sciences*, 11(12):7038-7047. DOI: 10.3390/ijms12107038

Calandra, T., Roberts, J.A., Antonelli, M., Bassetti, M. & Vincent, J.L. 2016. Diagnosis and management of invasive candidiasis in the ICU: an updated approach to an old enemy. *Critical care,* 20:125.
DOI: 10.1186/s13054-016-1314-6

Carmello, J.C., Alves, F., Basso, F.G., De Souza Costa, C.A., Bagnato, V.S., De Oliveira Mima, E.G. & Pavarina, A.C. 2016. Treatment of oral candidiasis using photodithazine1- mediated photodynamic therapy in vivo. *PloS one,* 11(6):e0156947. DOI: 10.1371/journal.pone.0156947

Casadevall, A. & Pirofski, L. 2007. Accidental virulence, cryptic pathogenesis, martians, lost hosts and the pathogenicity of environmental microbes. *Eukaryotic cell,* 6(12):2169-2174. DOI: 10.1128/EC.00308-07

Chopra, I. & Roberts, M. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology bacterial resistance. *American society for microbiology,* 65(2):232-260. DOI: 10.1128/MMBR.65.2.232-260.2001

Clardy, J. & Walsh, C. 2004. Lessons from natural molecules. *Nature*, 432:829-837. DOI: 10.1038/nature03194

Cohen, J. 1969. Statistical power of analysis for behavioural sciences. 2nd ed. Hillsdale, NJ: Erlbaum.

Colombo, A.L., Nucci, M., Park, B.J., Nouér, S.A., Arthington-Skaggs, B. & Da Matta, D.A. 2006. Epidemiology of candidaemia in Brazil: a nationwide sentinel surveillance of candidaemia in eleven medical centres. *Journal of clinical microbiology,* 44(8):2816-2823. DOI: 10.1128/JCM.00773-06

Concia, E., Azzini, A.M. & Conti, M. 2009. Epidemiology incidence & risk factors for invasive candidiasis in high risk patients. *Drugs*, 69(1):5-14. DOI: 10.2165/11315500-000000000-00000

Cornely, O.A., Lass-Flörl, C., Lagrou, K., Arsic-Arsenijevic, V. & Hoenigl., M. 2017. Improving outcome of fungal diseases – Guiding experts and patients towards excellence. *Mycoses,* 60(7):420-425. DOI: 10.1111/myc.12628l

Curtis, J.R. 1998. The 'patient-centred' outcomes of critical care: what are they and how should they be used? *New horizons,* 6(1):26-32. PMID:9508255

Davis, B.D. 1987. *Mechanism* of bactericidal action of aminoglycosides. *Microbiol*ogy, 51(3):341-350. PMCID: PMC373115

De Waele, J.J., Vogelaers, D., Blot, S. & Colardyn, F. 2003. Fungal infections in patients with severe acute pancreatitis and the use of prophylactic therapy. *Critical care,* 7(Suppl 2):P130. DOI: 10.1186/cc2019

DoH ***see*** South Africa. DoH.

Drlica, K. & Zhao, X. 1997. DNA Gyrase, topoisomerase IV, and the 4-Quinolones. *Microbiology and molecular biology reviews,* 61(3):377-392.

Droege, M.E., Van Fleet, S.L. & Meuller, E.W. 2016. Application of antibiotic pharmacodynamics and dosing principles in patients with sepsis. *Critical care nurse,* 36(2):22-32. DOI: 10.4037/ccn2016881

Eggimann, P. & Pittet, D. 2014. *Candida* colonization index and subsequent infection in critically ill surgical patients: 20 years later. *Intensive care medicine,* 40(10):1429-1448. DOI: 10.1007/s00134-014-3355-z

Eggimann, P., Bille, J. & Marchetti, O. 2011. Diagnosis of invasive candidiasis in the ICU. *Annals of intensive care,* 1(1):1-37. DOI: 10.1186/2110-5820-1-37

Eggimann, P., Gerbino, J. & Pitter, D. 2003. Epidemiology of *Candida* species infection in critically ill non-immunosuppressed patients. *The Lancet infectious diseases*, 3(11):685-702. DOI: 10.1016/S1473-3099(03)00801-6

Fischbach, M.A. & Walsh, C.T. 2009. Antibiotics for emerging pathogens. *Science,* 325(5944):1089-1093. DOI: 10.1126/science.1176667

Flanagan, P.G. & Barnes, R.A. 1998. Fungal infection in the intensive care unit. *Journal of hospital infection,* 38(3):163-77. DOI: 10.1016/S0195-6701(98)90271-7

Fridkin, K. & Jarvis, W.R. 1996. Epidemiology of nosocomial fungal infections. *Clinical microbiology reviews,* 9(4):499-511.

Fronczek, J., Polok, K.J., Nowak-Kózka, I., Władarczyk, A., Górka, J., Czuczwar, M., Krawczyk, P., Ziętkiewicz, M., Nowak, Ł.R., Żukowski, M., Kotfis, K., Cwyl, K., Gajdosz, R., Bohatyrewicz, R., Biernawska, J., Grudzień, P., Nasiłowski, P., Popek, N., Cyrankiewicz, W., Wawrzyniak, K., Wnuk, M., Maciejewski, D., Studzińska, D., Bernas, S., Piechota, M., Machała, W., Serwa, M., Wujtewicz, M., Stefaniak, J., Szymkowiak, M., Gawda, R., Adamik, B., Kozera, N., Goździk, W., Flaatten, H. & Szczeklik, W. 2018. Frailty is associated with an increated mortality among patients ≥ 80 years old treated in Polish ICU`s. *Anaesthesiol intensive therapy*, 50(4):245-251.

Frost, K.J. 2007. An overview of antibiotic therapy. Nursing standard, 22(9):51-57). DOI: 10.7748/ns2007.11.22.9.51.c6235

Gallis, H.A., Drew, R.H. & Pickard, W.W. 1990. Amphotericin B: 30 years of clinical experience. *Reviews of infectious diseases*, 12(2):308-329. DOI: 10.1093/clinids/12.2.308

Gearing, R.E., Mian, I.A., Barber, J. & Ickowicz, A. 2006. A methodology for conducting retrospective chart review research in child and adolescent psychiatry. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, 15(3):126-134. PMCID: PMC2277255

Giri, S. & Kindo, A.J. 2012. A review of *Candida* species causing blood stream infection. *Indian journal of medical microbiology*, 30(3):270-278.

Glazier, A.M., Nadeau, J.H. & Aitman, T.J. 2002. Finding genes that underlie complex traits. *Science,* 298(5602):2344-2349. DOI: 10.1126/science.1076641

Gloor, B., Muller, C.A. & Worni, M. 2001. Pancreatic infection in severe pancreatitis: the role of fungus and multi-resistant organisms. *Journal of the American Medical Association*, 136(5):592-596. DOI: 10.1001/archsurg.136.5.592

Gong, X., Luan, T., Wu, X., Li, G., Qiu, H., Kang, Y., Qin, B., Fang, Q., Cui, W., Qin, Y., Li, J. & Zang, B. 2016. Invasive candidiasis in intensive care units in China: risk factors and prognoses of *Candida albicans* and non–albicans *Candida* infections. *American journal of infection control*, 44(5):e59-e63. DOI: 10.1016/j.ajic.2015.11.028

Gow, N.A.R. & Yadav, B. 2017. Microbe profile: *Candida albicans:* a shape-changing, opportunistic pathogenic fungus of humans. *Microbiology*, 163:1145-1147. DOI: 10.1099/MIC.0.000499

Gregory, K.E. & Radovinsky, L. 2012. Research strategies that result in optimal data collection from the patient medical record. *Applied nursing research journal,* 25(2):108-116. DOI: 10.1016/j.apnr.2010.02.004

Grewe, M., Tsiotos, G.G., De-Leon, E.L. & Sarr, M.G. 1999. Fungal infection in acute necrotising pancreatitis. *American college of surgeons*, 188(4):408-414. DOI: 10.1016/S1072-7515(98)00334-2

Groll, A.H., Piscitelli, S.C. & Walsh, T.J. 1998. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. *Advanced pharmacology*, 44:343-500. PMID: 9547888

Grubb, S.E.W., Murdoch, C., Sudbery, P.E., Saville, S.P., Ribot, J.L. & Thornhill, M.H. 2008. *Candida albicans* -endothelial cell interactions: a key step in the pathogenesis of systemic candidiasis*. Infection and immunity*, 76(10):4370-4377. DOI: 10.1128/IAI.00332-08

Guery, B.P., Arendrup, G.A., Auzinger, G., Azoulay, E., Borges, M., Johnson, E.M., Müller, E., Putensen, C., Rostein, C., Sganga, G., Venditti, M., Zaragozr, R.C. & Kullberg, B.J. 2009. Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: part II. Treatment*. Intensive care medicine*, 35:206-214. DOI: 10.1007/s00134-008-1339-6

Heimann, S.M., Cornely, O.A., Wisplinghoff, H., Kochanek, M., Stippel, D. & Padosch, S.A. 2014. Candidaemia in the intensive care unit: analysis of direct treatment costs and clinical outcome in patients treated with echinocandins or fluconazole. *European journal of clinical microbiological infectious diseases*, 34(2):331-338. DOI: 10.1007/s10096-014-2230-8

Hickman, L. & Disler, R. 2016. Quantitative research. (*In* Borbasi, S. & Jackson, D. *eds.* Navigating the maze of research: enhancing nursing and midwifery practice. 4th ed. Chatswood: Elsevier. p. 103-142).

Ho, K. & Cheng, T. 2010. Common superficial fungal infections: a short review. *Medical bulletin*, 15(11):23-27.

Hogan, D.A. & Kolter, R. 2002. *Pseudomonas-Candida* interactions: an ecological role for virulence factors. *Science*, 296(5576):2229-2232. DOI: 10.1126/science.1070784

Hollenbach, E. 2008. Invasive candidiasis in the ICU: evidence based and on the edge of evidence. *Mycoses*, 51(2):25-45. DOI: 10.1111/j.1439-0507.2008.01571.x/full

Horn, D.L., Neofytos, D., Anaissie, E.J., Fishman, J.A., Steinbach, W.J., Olyaei, A.J., Marr, K.A., Pfaller, M.A., Chang, C.H. & Webster, K.M. 2009. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. *Clinical infectious diseases*, 48(12):1695-1703. DOI: 10.1086/599039

Ibrahim, O.M. & Polk, R.E. 2014. Antimicrobial use metrics and benchmarking to improve stewardship outcomes. *Infectious disease clinics*, 28:194-214

Istúriz, R.E. & Carbon, C. 2000. Antibiotic use in developing countries. *Infectious control and hospital epidemiology*, 21(6):394-403. DOI: 10.1086/501780

Jacobs, D.M., Beyda, N.D., Asuphon, O., Alam, M.J. & Garey, K.W. 2015. Host factors and clinical outcomes of *Candida* colonization in critically ill patients. *Mycopathologia,* 179(2):87-93. DOI: 10.1007/s11046-014-9809-6

Karabinis, A., Hill, C., Leclerq, B., Tancrede, C., Baume, D. & Andremont, A. 1988. Risk factors for candidaemia in cancer patients: a case-control study. *Journal of clinical microbiology,* 26(3):429-432.

Kautzky, A., Staudinger, T. & Presterl, E. 2015. Invasive *Candida* infections in patients of a medical intensive care unit. *The central European journal of medicine,* 127(3-4):132-142. DOI: 10.1007/s00508-014-0644-z

Kalan, L. & Grice, E. 2017. Fungi in the wound microbiome. *Advances in wound care,* 7(7):247-255.

Kett, D.H., Azoulay, E., Echeverria, P.M. & Vincent, J.L. 2011. *Candida* bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. *Critical care medicine*, 39(4):665-670. DOI: 10.1097/CCM.0b013e318206c1ca

Kim. J. & Sudberry, P. 2011. *Candida albicans*, a major human fungal pathogen. *Journal of microbiology*, 49(2):171-177. DOI: 10.1007/s12275-011-1064-7

Klotz, S.A., Gaur, N.K., De, A.R., Sheppard. D. & Khardori, N. 2007. *Candida albicans* proteins mediate aggregation with bacteria and yeasts. *Medical mycology*, 45(4):363-370. DOI: 10.1080/13693780701299333

Kohanski, M.A., Dwyer, D.J., Hayete, B., Lawrence, C.A. & Collins, J.C. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell,* 130(5):797-810. DOI: 10.1016/j.cell.2007.06.049.

Kojic, E.M. & Darouiche, R.O. 2004. *Candida* Infections of medical devices. *Clinical microbiology reviews*, 17(2):255-267. DOI: 10.1128/CMR.17.2.255-267.2004

Kollef, M.D. & Fraser, V.J. 2001. Antibiotic resistance in the intensive care unit. *Annals of internal medicine,* 134(4):298-314. DOI: 10.7326/0003-4819-134-4-200102200-00014

Kullberg, B.J. & Arendrup, M.C. 2016. Invasive Candidiasis. *New England journal of medicine,* 374:793-795. DOI: 10.1056/NEJMc/1514201.

Kurtzman, C.P., Sunnerhagen, P. & Piškur, J. 2006. Taxonomy and phylogenetic diversity among the yeasts: comparative genomics: using fungi as models. (*In* Sunnerhagen, P. & Piskur, J., *eds*. Topics in current genetics, vol. 15. Berlin: Springer-Verlag. p. 29-46).

Lass-Flörl, C. 2009. The changing face of epidemiology of invasive fungal disease in Europe. *Mycoses,* 52(3):197-205. DOI: 10.1111/j.1439-0507.2009.01691.x/full

León, C., Ruiz-Santana, S., Saavedra, P., Almirante, B., Nolla-Salas, J., Álvarez-Lerma, F., Garnacho-Montero, J. & León, M.A. 2006. A bedside scoring system ("*Candida* score') for early antifungal treatment in nonneutropenic critically ill patients with *Candida* colonization. *Critical care medicine,* 34(3):730-737. DOI: 10.1097/01.CCM.0000202208.37364.7D

Leroy, O., Gangneux, J.P., Montravers, P., Mira, J.P., Gouin, F., Sollet, J.P., Carlet, J., Reynes, J., Rosenheim, M., Regnier, B. & Lortholary, O. 2009. Epidemiology, management, and risk factors for death of invasive *candida* infections in critical care: a multicentre, prospective, observational study in France. *Society of critical care medicine*, 37(5):1612-1618. DOI: 1097/CCM.0b013e31819efac0

Livermore, D.M. 2012. Current epidemiology and growing resistance of gram negative pathogens. *Korean journal of internal medicine,* 27(2):128-142. DOI: 10.3904/kjim.2012.27.2.128

Lortholary, O., Renaudat, C., Sitbon, K., Madec, Y., Denoeud-Ndam, L. & Wolff, M. 2014. Worrisome trends in incidence and mortality of candidaemia in intensive care units. *Intensive care medicine,* 40(9):1303-1312. DOI: 10.1007/s00134-014-3408-3

Lund, B.C., Carrel, M., Gellad, W., Chrischilles, A. & Kaboli, P.J. 2015. Incidence- versus prevalence-based measure in the veterans' health administration. *The American geriatrics society,* 63(8):1601-1607. DOI: 10.1111/jgs.13560

Magambo, B., Nazziwa, J., Bbosa, N., Gupta, R.K., Kaleebu, P. & Parry, C.M. 2014. The arrival of untreatable multidrug-resistant HIV-1 in sub-Saharan Africa. *AIDS*, 28(9):1373-1374.

Mahadevan, S., Abdullah, A. & Ponnan, R. 2014. Cost analysis on ERP system implementation amongst Malaysian SMEs. *International journal of trade, economics and finance,* 5(1):72-76. DOI: 10.7763/IJTEF.2014.V5.343

Mann, C.J. 2003. Observational research methods: research design II: cohort, cross sectional and case-control studies. *Emergency medical journal,* 20(1):54-60.

Manolakaki, D., Velmahos, G., Kourkoumpetis, T., Chang, Y., Alam, H.B., De Moya, M.M. & Mylonakis, E. 2010. *Candida* infection and colonization among trauma patients. *Virulence*, 1(5):367-375. DOI: 10.4161/viru.1.5.12796

Martinez, J.L. 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental pollution*, 157(11):2893-2902. DOI: 10.1016/j.envpol.2009.05.051

Mayan, M.J. 2001. An introduction to qualitative methods: a training module for students and professionals. Edmonton, Alberta: International Institute for Qualitative Methodology.

Mayer, G. 2016. Immunology – chapter one: Innate (non-specific) immunity. (*In* Hunt, R., *ed.* Microbiology and immunology on-line. University of South Carolina. School of Medicine). http://www.microbiologybook.org/ghaffar/innate.htm Date of access: 15 Feb. 2017.

McCullough, M.J., Ross, B.C. & Reade, P.C. 1996. *Candida* albicans: a review of its history, taxonomy, virulence attributes, and methods of strain differentiation. *International journal of oral maxillofacial surgery,* 25(2):136-144. DOI: 10.1016/S0901-5027(96)80060-9

Menon, G.R. & Cash, R. 2006. Research involving medical records review: an Indian perspective. *Indian journal of medical ethics,* 3(2):2523-2531.

Miller-Keane, B.F. & O'Toole, M. 2005. Encyclopaedia and dictionary of medicine, nursing, and allied health. 7th ed. Philadelphia, PA: Saunders.

Momattin, H., Anfal, Y.A., Khurram, M. & Jaffar, A.A. 2018. Benchmarking of antibiotic usage: An adjustment to reflect antibiotic stewardship program in Saudi Arabia. *Journal of infection and public health*, 11(3):310-313.

Moore, R.T. 1980. Taxonomic proposals for the classification of marine yeasts and other yeast-like fungi including the smuts. *Botanica marina*, 23(6):361-373.

Mosby. 2009. Mosby's dictionary of medicine, nursing & health professions. St. Louis, MO: Mosby/Elsevier.

Mouton, J.W. 2012. The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. *Clinical microbiology & infection*, 18(3):37-45. DOI: 10.1111/j.1469-0691.2011.03752.x

Mouton, J.W. & Marais, H.C. 1994. Basic concepts in the methodology of the social sciences. Pretoria: Human Sciences Research Council.

Moyes, D.L., Runglal, M., Murciano, C., Shen, C., Nayar, D., Thavaraj, S., Kohli, A., Islam, A., Mora-Montes, H. & Challacombe, S.J. 2010. A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of *Candida albicans* in epithelial cells. *Cell host & microbe*, 8(3):225-235. DOI: 10.1016/j.chom.2010.08.002

Muilwijk, E.W., Schouten, J.A., van Leeuwen, H.J., van Zanten, A.R.H., de Lange, D.W., Colbers, A., Verweij, P.E., Burger, D.M., Pickkers, P. & Brüggemann, J.M. 2014. Pharmacokinetics of caspofungin in ICU patients. *Journal of antimicrobial chemotherapy,* 69:3294-3299. DOI: 10.1093/jac/dku313

Muscedere, J, Rewa, O., Mckechnie, K., Jiang, X., Laporta, D. & Heyland, D.K. 2011. Subglottic secretion drainage for the prevention of ventilator-associated pneumonia: A systematic review and meta-analysis. *Critical care medicine,* 39:1985-1991.

Navarre, W.W. & Schneewind, O. 1999. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiology and molecular biology review,* 63(1):174-229. PM:10066836

Nieto-Rodríguez, J.A., Kusne, S., Manez, R., Irish, W., Linden, P., Magnone, M., Wing, E.J., Fung, J.J. & Staal, T. 1996. Factors associated with the development of Candidemia and Candidemia-related related deaths among liver transplant recipients. *Annals of surgery*, 223(1):70-76. PMCID: PMC1235065

Noni, M., Katelari, A., Athanasios, K., Theochari, L., Lympari, I., Alexandrou-Athanassoulis, H., Doudounakis, S. & Dimopoulos, G. 2015. *Candida albicans* chronic colonisation in cystic fibrosis may be associated with inhaled antibiotics. *Mycosis,* 58(7):416-421. DOI: 10.1111/myc.12338/full

Ntagiopoulos, P.G., Paramythiotou, E., Antoniadou, A., Giamarellou, H. & Karabinis, A. 2007. Impact of antibiotic restriction policy on the antibiotic resistance patterns of gram-negative microorganisms in an intensive care unit in Greece. *International journal of antimicrobial agents*, 30(4):360-365. DOI: 10.1016/j.ijantimicag.2007.05.012

Olaechea, P.M., Palomar, M., León-Gil, C., Álvarez-Lerma, C., Jordá, R., Nolla-Salas, J. & León-Regidor M.A. 2004. Economic impact of *Candida* colonization and *Candida* infection in the critically ill patient. *European journal of clinical microbiology and infectious diseases,* 23(4):323-330. DOI: 10.1007/s10096-004-1104-x

Pankey, G.A. & Sabath, L.D. 2004. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram-positive bacterial infections. *Clinical infectious diseases,* 38(6):864-870. DOI: 10.1086/381972

Papathomas, M. & Richardson, S. 2014. Exploring dependence between categorical variables: benefits and limitations of using variable selection within Bayesian clustering in relation to log-linear modelling with interaction terms. *Journal of statistical planning and inference,* 173(2016):47-63. DOI: 10.1016/j.jspi.2016.01.002

Pappas, P.G. 2006. Invasive candidiasis. *Clinical infectious diseases,* 20(3):485-506. DOI: 10.1016/j.idc.2006.07.004

Pappas, P.G., Kauffman, C.A., Andes, D.R., Clancy, C.J., Marr, K.A., Ostrosky-Zeichner, L., Reboli, A.C., Schuster, M.G., Vazquez, J.A., Walsh, T.J., Zaoutis, T.E. & Sobel, J.D. 2015. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. *Clinical infectious diseases,* 62(4):409-417. DOI: 10.1093/cid/civ933

Pappas, P.G., Rex, J.H., Lee, J., Hamill, R.J., Larsen, R.A., Powderly, W., Kauffman, C.A., Hyslop, N., Mangino, J.E., Chapman, S., Horowitz, H.W., Edwards, J.E. & Dismukes, W.E. 2003. A prospective observational study of candidaemia: epidemiology, therapy and influences on mortality in hospitalized adults and paediatric patients. *Clinical infectious diseases,* 37(5):634-643. DOI: 10.1086/376906

Pennisi, M. & Antonelli, M. 2009. Clinical aspects of invasive candidiasis in critically ill patients. *Drugs*, 69(Suppl 1):21-28. DOI: 10.2165/11315590-000000000-00000

Pfaller, M.A. & Diekema, D.J. 2007. Epidemiology of invasive candidiasis persistent public health problem. *Clinical microbiological reviews*, 20(1):133-163. DOI: 10.1128/CMR.00029-06

Pfaller, M.A., Diekema, D.J., Castanheira, M. & Jones, R.N. 2011. Definitions and epidemiology of *Candida* species not susceptible to echinocandins. *Current fungal infection reports*, 5(3):120-127. DOI: 10.1007/s12281-011-0053-y

Pinhati, H.S.M., Casulari, L.A., Souza, A.C.R., Siqueira, R.A., Damasceno, C.M.G. & Colombo, A.L. 2016. Outbreak of candidaemia caused by fluconazole resistant *Candida parapsilosis* strains in an intensive care unit. *BMC Infectious diseases,* 16(1):433.
DOI: 10.1186/s12879-016-1767-9

Pittet, D., Monod, M., Suter, P.M., Frenk, E. & Auckenthaler, R. 1994. *Candida* colonization and subsequent infections in critically ill surgical patients. *Annals of surgery*, 220(6):751-758. PMC1234477

Poehlsgaard, J. & Douthwaite, S. 2005. The bacterial ribosome as a target for antibiotics. *Nature reviews microbiology,* 3:870-881. DOI:10.1038/nrmicro1265.

Polit, F.P. & Beck C.T. 2014. Essentials of nursing research: appraising evidence for nursing practice. 8th ed. Philadelphia, PA: Lippincott Williams & Wilkins.

Prin, M., Harrison, D., Rowan, K. & Wunsch, H. 2015. Epidemiology of admissions to 11 stand-alone high dependency care units in the UK. *Intensive care medicine,* 41(11):1903-1910.
DOI: 10.1007/s00134-015-4011-y

Rajendran, R., Robertson, D.P., Hodge, P.J., Lappin, D.F. & Ramage, G. 2010. Hydrolytic enzyme production is associated with *Candida* albicans biofilm formation from patients with type 1 diabetes. *Mycopathologia*, 170(4):229-235. DOI: 10.1007/s11046-010-9319-0

Rentz, A.M. & Halpern, M. 1998. The impact of candidemia on length of hospital stay, outcome and, overall cost of illness. *Clinical infectious diseases society of America,* 27:781-788. DOI: 10.1086/514955

Reynolds, P.E. 1989. Structure, biochemistry and mechanism of action of glycopeptides antibiotics. *European journal of clinical microbiology and infectious diseases,* 8(11):943-950.

Richardson, J.P., Moyes, D.L., Ho, J. & Naglik, J.R. 2018. *Candida* innate immunity at the mucosa. *Seminars in cell & developmental biology,* 1:1-13.

Rinaldi, M.G. 1991. Problems with the diagnosis of invasive fungal diseases*. Infectious diseases review,* 13(3):493-495. DOI: 10.1093/clinids/13.3.493

Rodríguez-Rojas, A., Rodriguez-Beltran, J., Couce, A. & Blazquez, J. 2013. Antibiotics and antibiotic resistance: a bitter fight against evolution. *International journal of medical microbiology*, 303(6):293-297. DOI: 10.1016/j.ijmm.2013.02.004

Romani, L. 2008. Cell-mediated immunity to fungi: a reassessment. *Medical mycology,* 46(6):515-529. DOI: 10.1080/13693780801971450

Sardi, J.C.O., Scorzoni, T., Bernardi, A.M. & Mendes Giannini, M.J.S. 2013. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of medical microbiology*, 62(1):10-24. DOI:10.1099/jmm.0.045054-0

Saville, S.P., Lazzell, A.L., Monteagudo, C. & Lopez-Ribot, J.L. 2003. Engineered control of cell morphology *in vivo* reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryotic cell*, 2(5):1053-1066. DOI: 10.1128/EC.2.5.1053-1060.2003

Schaal, J.V., Leclerc, T., Soler, C., Donat, N., Cirrode, A., Jault, P. & Bargues, L. 2015. Epidemiology of filamentous fungal infections in burned patients: a French retrospective study. *Burns,* 41(4):853-863. DOI: 10.1016/j.burns.2014.10.024

Schmidt, C.O. & Kohlmann, T. 2008. When to use the odds ratio or the relative risk? *International journal of public health*, 53(3):165-167. DOI: 10.1007/s00038-008-7068-3

Shapiro, R.S., Robbins, N. & Cowen, L.E. 2011. Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiology & molecular biology*, 75(2):213-267. DOI: 10.1128/MMBR.00045-10

Shareck, J. & Belhumeur, P. 2011. Modulation of morphogenesis in *Candida albicans* by various small molecules. *Eukaryotic cell*, 10(8):1004-1012. DOI: 10.1128/EC.05030-11

Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D.W. & Azeredo, J. 2011. *Candida glabrata, Candida parapsilosis and Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS microbiology*, 36(2):288-305. DOI: 10.1111/j.1574-6976.2011.00278.x

South Africa. DoH. 2015. Ethics in health research: principles, processes and structures. 2nd ed. Pretoria: Department of Health. http://www0.sun.ac.za/research/assets/files/Integrity_and_Ethics/DoH%202015%20Ethics%20in%20Health%20Research%20-%20Principles,%20Processes%20and%20Structures%202nd%20Ed.pdf Date of access: 26 Jun 2016

Tacconelli, E., Müller, N.F., Lemmen, S., Mutter, N.T., Hagel, S. & Meyer, E. 2016. Infection risk in sterile operative procedures. *Deutsches Ärzteblatt International,* 113(16):271-278. DOI: 10.3238/arztebl.2016.0271

Thompson, H.S. & Webb, A.W., *eds.* 2014. Contrast media: medical radiology: diagnostic imagining. 3rd ed. Berlin: Springer.

Trevor, A.J., Katzung, B.G. & Masters, S.B. 2005. Pharmacology examination & broad review. 9th ed. New York, NY: McGraw-Hill Medical Publishing.

Uhl, M.A., Biery, M., Craig, N. & Johnson, A.D. 2003. Haploinsufficiency-based largescale forward genetic analysis of filamentous growth in the diploid human fungal pathogen *C. albicans*. *European molecular biology organization*, 22(11):2668-2678. DOI: 10.1093/emboj/cdg256

Van Burik, J.H. & Magee, P.T. 2001. Aspects of fungal pathogenesis in humans. *Annual revision of microbiology,* 55(1):743-772. DOI: 10.1146/annurev.micro.55.1.743

Vazquez, J.A. 2010. Invasive fungal infections in the intensive care unit. *Seminars in respiratory critical care medicine,* (31)1:79-86. DOI: 10.1055/s-0029-1246289

Vincent, J.L., Rello, J., Marshall, J., Silva, E. & Anzueto, A. 2009. International study of the outcomes of intensive care units. *Journal of the American Medical Association*, 302(21):2323-2329. DOI: 10.1001/jama.2009.1754

Walsh, C. 2000. Molecular mechanisms that confer antibacterial drug resistance. *Nature,* 406:775-781. doi:10.1038/35021219

Weisblum, B. & Davies, J. 1968. Antibiotic inhibitors of the bacterial ribosome. *Bacteriology reviews,* 32(4):493-528. PMCID:PMC413162.

Wenham, T. & Pittard, A. 2009. Intensive care unit environment. *Critical care & pain*, 9(6):178-183. DOI: 10.1093/bjaceaccp/mkp036

Wey, S.B., Motomi, M. & Pfaller, M. 1988. Hospital-acquired candidaemia: the attributable mortality and excess length of stay. *Archives of internal medicine,* 148(12):2642-2645. DOI: 10.1001/archinte.1988.00380120094019

Whittington, A., Gow, N.A.R. & Hube, B. 2014. From commensal to pathogen: *Candida albicans*. (*In* Kurzai O., *ed.* Human fungal pathogens: the Mycota. Berlin: Springer, p. 12:3-18). (A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research, vol 12),

Wisplinghoff, H., Bischoff, T., Tallent, S.M., Seifert, H., Wenzel, R.P. & Edmond, M.B. 2004. Nosocomial bloodstream infections in hospital analysis of 24 179 cases from prospective nationwide surveillance study. *Clinical infectious diseases*, 39(3):309-317. DOI: 10.1086/421946

Wood, J.M. & Ross-Kerr, J.C. 2011. Basic steps in planning nursing research: from question to proposal. 7th ed. Sudbury: Jones and Bartlett.

Worster, A. & Haines, T. 2004. Advanced statistics: understanding medical record review (MRR) studies. *Academic emergency medicine*, 11(2):187-192. DOI: 10.1111/j.1553-2712.2004.tb01433.x

Yapar, N. 2014. Epidemiology and risk factors for invasive candidiasis. *Therapeutics and clinical risk management*, 10:95-105. PMC3928396

ANNEXURES

ANNEXURE A: DATA COLLECTION TOOL – ANTIBIOTIC TREATMENT

Number	Admission date	Release date	Gender (Male=1 Female=2)	Age	Diagnosis (Primary *Candida*=1 Secondary *Candida*=2)	Antibiotic use (No=1 Yes=2)	Antibiotic duration (All) (<7 days=1 ≥7 days=2)	Single antibiotic use (No=1 Yes=2)	Manufacturer dose indication (MDI) vs Non-MDI (MDI=1 Non-MDI=2)	Manufacturer dosage interval (No = 1 Yes=2)	Route of administration (Oral=1 IV=2)

ANNEXURE B: DATA COLLECTION TOOL – ANTIFUNGAL TREATMENT

Number	Admission date	Release date	Gender (Male=1 Female=2)	Age	Diagnosis (Primary *Candida*=1 Secondary *Candida*=2)	Antifungal use (No=1 Yes=2)	MDI vs Non-MDI (MDI=1 Non-MDI=2)	Manufacturer dosage interval (No = 1 Yes=2)	Route of administration (Oral=1 IV=2)

ANNEXURE C: DATA COLLECTION TOOL – TREATMENT

1. Active ingredient

2. Dose

3. Indication

4. Manufacturer dosage indication used (MDI) - (yes = 0, higher = 1, lower= 2)

	Penicillin				Cephalosporin				Fluoroquinolones				Aminoglycosides				Monobactam				Carbapenem				Macrolide				Other			
Number	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**

ANNEXURE D: PERMISSION

10 January 2017

Dear Me A Pieterse

RE: PERMISSION TO CONDUCT RESEARCH

Hereby we grant permission to conduct your research regarding "Antibiotic exposure as a risk factor for secondary *Candida* infections in a private hospital intensive care unit" at

It is very important that the name of the Hospital or any of its wards as well as personal information regarding patients, doctors and staff are not to be divulged at any time. A confidential agreement to this effect must be signed.

We wish you all the best with your research.

Kind regards

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Faculty of Health Sciences Ethics Office for Research, Training and Support

Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

08 November 2017

Dr JM du Plessis
Pharmacy practice
MUSA

Dear Dr du Plessis

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00040-17-S1

Kindly use the ethics reference number provided above in all future correspondence or documents submitted to the administrative assistant of the Health Research Ethics Committee (HREC) secretariat.

Study title: Antibiotic exposure as a risk factor for secondary Candida infections in a private hospital intensive care unit

Study leader/Researcher: Dr JM du Plessis

Student: AM Pieterse

Application type: Single study

Risk level: Minimal (monitoring report required annually)

You are kindly informed that your ethics approval application has been successful and fulfils all requirements for approval. Your study is approved for a year and may commence from 08/11/2017. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation. A monitoring report should be submitted two months prior to the reporting dates as indicated i.e. annually for minimal risk studies, six-monthly for medium risk studies and three-monthly for high risk studies, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at

Ethics-HRECMonitoring@nwu.ac.za. Annually, a number of studies may be randomly selected for an internal audit.

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. These requests should be submitted to Ethics-HRECApply@nwu.ac.za with a cover letter with a specific subject title indicating, "Amendment request: NWU-XXX-XXX". The letter should include the title of the approved study, the names of the researchers involved, the nature of the amendment/s being made (indicating what changes have been made as well as where they have been made), which documents have been attached and any further explanation to clarify the amendment request being submitted. The amendments made should be indicated in yellow highlight in the amended documents. The *e-mail*, to which you attach the documents that you send, should have a specific subject line indicating that it is an amendment request as well as the nature of the amendment e.g. "Amendment request: NWU-XXX-XXX". This submission will be handled via the expedited process.

Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form to Ethics-HRECIncident-SAE@nwu.ac.za. The *e-mail*, to which you attach the documents that you send, should have a specific subject line indicating that it is a notification of a serious adverse event or incident in a specific project e.g. "SAE/Incident notification: NWU-XXX-XXX". Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Prof Wayne Towers
HREC Chairperson

Prof Minrie Greeff
Ethics Office Head

ANNEXURE E: CONFIDENTIALITY UNDERTAKING

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

CONFIDENTIALITY UNDERTAKING

entered into between:

I, the undersigned

Prof / Dr / Mr / Ms ANNA MARIA PIETERSE

Identity Number 7709010048086

Address 29 WESTINGHOUSE STR, SONNEVELD, BRAKPAN, 1540

hereby undertake in favor of WILMED PARK HOSPITAL

Address Cnr Ametis Street & Philip Gerber Crescent, Wilkoppies, Klerksdorp, 2571

1 Interpretation and definitions

1.1 In this undertaking, unless inconsistent with, or otherwise indicated by the context

1.1.1 "Confidential Information" shall include all information that is confidential in its nature or marked as confidential and shall include any existing and new information obtained by me after the Commencement Date, including but not be limited in its interpretation to, research data, information concerning research participants, all secret knowledge, technical information and specifications, manufacturing techniques, designs, diagrams, instruction manuals, blueprints, electronic artwork, samples, devices, demonstrations, formulae, know-how, intellectual property, information concerning materials, marketing and business information generally, financial information that may include remuneration detail, pay slips, information relating to human capital and employment contract: employment conditions, ledgers, income and expenditures and other materials of whatever description in which Wilmed Park Hospital has an interest in being kept confidential; and

1.1.2 "Commencement Date" means the date of signature of this undertaking by myself.

1.2 The headings of clauses are intended for convenience only and shall not affect the interpretation of this undertaking.

2 Preamble

2.1 In performing certain duties requested by Wilmed Park Hospital, I will have access to certain Confidential Information provided by Wilmed Park Hospital in order to perform the said duties and I agree that it must be kept confidential.

1

2.2 Wilmed Park Hospital has agreed to disclose certain of this Confidential Information and other information to me subject to me agreeing to the terms of confidentiality set out herein.

3 Title to the Confidential Information

I hereby acknowledge that all right, title and interest in and to the Confidential Information vests in Wilmed Park Hospital and that I will have no claim of any nature in and to the Confidential Information.

4 Period of confidentiality

The provisions of this undertaking shall begin on the Commencement Date and remain in force indefinitely.

5 Non-disclosure and undertakings

I undertake:

5.1 to maintain the confidentiality of any Confidential Information to which I shall be allowed access by Wilmed Park Hospital, whether before or after the Commencement Date of this undertaking. I will not divulge or permit to be divulged to any person any aspect of such Confidential Information otherwise than may be allowed in terms of this undertaking;

5.2 to take all such steps as may be necessary to prevent the Confidential Information falling into the hands of an unauthorised third party;

5.3 not to make use of any of the Confidential Information in the development, manufacture, marketing and/or sale of any goods;

5.4 not to use any research data for publication purposes;

5.5 not to use or disclose or attempt to use or disclose the Confidential Information for any purpose other than performing research purposes only and includes questionnaires, interviews with participants, data gathering, data analysis and personal information of participants/research subjects;

5.6 not to use or attempt to use the Confidential Information in any manner which will cause or be likely to cause injury or loss to a research participant or Wilmed Park Hospital; and

5.7 that all documentation furnished to me by Wilmed Park Hospital pursuant to this undertaking will remain the property of Wilmed Park Hospital and upon the request of Wilmed Park Hospital will be returned to Wilmed Park Hospital. I shall not make copies of any such documentation without the prior written consent of Wilmed Park Hospital.

6 Exception

The above undertakings by myself shall not apply to Confidential Information which I am compelled to disclose in terms of a court order.

7 Jurisdiction

This undertaking shall be governed by South African law be subject to the jurisdiction of South African courts in respect of any dispute flowing from this undertaking.

8 Whole agreement

8.1 This document constitutes the whole of this undertaking to the exclusion of all else.

8.2 No amendment, alteration, addition, variation or consensual cancellation of this undertaking will be valid unless in writing and signed by me and Wilmed Park Hospital.

Dated at ~~Pietermaritzburg~~ BRAEPARK this 27th January 2017

Witnesses:

1 .....

2 .....

(Signatures of witnesses)

.....

(Signature)

3

ANNEXURE F: CODE OF CONDUCT

CODE OF CONDUCT FOR RESEARCHERS

This code of conduct is applicable to all NWU researchers.

As a researcher of the North-West University (NWU), I subscribe to the rules of the NWU Institutional Research Ethics Regulatory Committee (IRERC), all applicable policies of the NWU as well as all national and international laws and regulations applicable to my field of study. Furthermore, I commit myself to abide by the ethical principles and responsibilities as set out in the Singapore statement on Research Integrity (22 September 2010), in any and all research endeavours that I undertake as a researcher of the NWU.

The four major principles of research integrity to which I will adhere and that will guide my research are:

- Honesty in all aspects of research
- Accountability in the conduct of research
- Professional courtesy and fairness in working with others
- Good stewardship of research on behalf of others

Consequently I will also adhere to the following ethical responsibilities:

1. I will take responsibility for the originality and trustworthiness of my research.

2. I will stay abreast of and adhere to all institutional, national, and international laws, regulations, and policies applicable and related to my research.

3. I will at all times employ appropriate research methods, base my conclusions on critical analysis of the evidence and report my findings and interpretations fully and objectively.

4. I will keep clear and accurate records of all research that I have conducted in a manner that will allow verification and replication of my work by others, if applicable.

5. I will, where applicable, share my data and findings openly and promptly, in line with external funding rules. This will be done as soon as possible after I have had an opportunity to establish priority and ownership claims.

6. I will take responsibility for my own contributions to publications, funding applications, reports and other representations of my research. I will also and only include authors who meet valid authorship criteria.

7. I will acknowledge the names and roles of those who made significant contributions to my research in publications, including writers, funders, sponsors, and others, but do not meet authorship criteria.

8. In my peer reviews, I will provide fair, prompt and rigorous evaluations and I will respect confidentiality when I review others' work.

9. I will disclose all conflicts of interest (financial and other) that could compromise the trustworthiness of my work in research proposals, publications, public communications, and in review activities.

10. When I publically address a community in the spirit of academic freedom, I will in all stages base my professional comments on research findings (if applicable) and my expertise. I will distinguish between professional comments and opinions based on personal views.

11. Should any irresponsible research practices and/or research misconduct become known to me or brought under my attention, I will report such irresponsible research activities to the appropriate authorities.

12. I will respond to irresponsible research practices or conduct, by taking prompt actions as set out in the procedures of the university. I will also protect those who report misconduct in good faith, to the best of my abilities.

13. I will endeavour to create and sustain an environment that encourage research integrity through education of students, research teams and peers, as well as abide by policies, and reasonable standards for advancement.

14. I will at all times weigh societal benefits against the risks inherent in my work.

Name: Anna Maria Pieterse Signature: Date: 27.06.20[illegible]

ANNEXURE G: ANTIBIOTIC CLASSIFICATION AND WORKING MECHANISM

ANTIBACTERIAL CLASS	ANTIMICROBIAL THERAPY	GENERAL WORKING MECHANISM
BETA-LACTAM / PENICILLIN		
Penicillin / Beta-Lactam	• Amoxicillin • Ampicillin • Apalcillin • Azlocillin • Bacampicillin • Benzathine • Benzylpenicillin • Carbenicillin • Cloxacillin • Dicloxacillin • Flucloxacillin • Mecillinam • Methicillin • Mezlocillin • Nafcillin • Oxacillin • Penicillin G • Penicillin V • Phentoxypenicillin • Piperacillin • Procaine Penicillin • Temocillin • Ticarcillin	Penicillin is bactericidal, acting by inhibition of the cell wall synthesis in susceptible organisms
ANTIBACTERIAL CLASS	**ANTIMICROBIAL THERAPY**	**GENERAL WORKING MECHANISM**
CEPHALOSPORINS		
First-generation Cephalosporin	• Cefacetrile • Cefadroxil • Cefalexin	Broad-spectrum semi-synthetic beta-lactam

ANTIBACTERIAL CLASS	ANTIMICROBIAL THERAPY	GENERAL WORKING MECHANISM
	• Cefatrizine • Cefazaflur • Cefazolin	antibiotics with similar working mechanism to that of penicillin
Second-generation Cephalosporin	• Cefaclor • Cefamandole • Cefotetan • Cefprozil • Cefuroxime	
Third-generation Cephalosporin	• Cefixime • Cefmenoxime • Cefodizime • Cefoperazone • Ceftazidime • Ceftriaxone	
Fourth-generation Cephalosporin	• Cefepime • Cefozopran • Cefpirome • Ceftaroline • Ceftobiprole • Fosamil	
CARBACEPHEM		
Carbacephem	• Loracarbef	Structurally related to cephalosporin with similar activity to second-generation cephalosporin.
CARBAPENEMS		
Carbapenems	• Artapenem • Doripenem • Imipenem • Meropenem • Panipenem	Broad-spectrum beta-lactam antibiotics

ANTIBACTERIAL CLASS	ANTIMICROBIAL THERAPY	GENERAL WORKING MECHANISM
MONOBACTAMS		
Monobactam	• Aztreonam • Carumonam • Nocardicin A • Tigemonam	Beta-lactam antibiotic with activity against aerobic Gram-negative organisms only
***B*-LACTAMASE INHIBITORS**		
Penams &Clavam	• Clavulanic acid • Sulbactam • Tazobactam	*β*-lactamase inhibitors
MACROLIDES, KETOLIDES AND LINCOSAMIDES		
Macrolides	• Azithromycin • Clarithromycin • Dirithromycin • Erythromycin • Roxithromycin	Macrolides bind to the bacterial 50S ribosomal subunit, inhibiting protein synthesis and consequently cell growth
Ketolides	• Telithromycin	
Lincosamides	• Clindamycin • Lincomycin	
TETRACYCLINE		
	• Doxycycline • Lymecycline • Minocycline • Oxytetracycline • Tetracycline	Broad-spectrum Bacteriostatic agents that inhibit protein synthesis by binding to 30S ribosomal subunits in the susceptible organism

ANTIBACTERIAL CLASS	ANTIMICROBIAL THERAPY	GENERAL WORKING MECHANISM
AMPHENICOL		
	• Chloramphenicol	Broad-spectrum – mainly bacteriostatic antibiotic
ANTIBACTERIAL CLASS	**ANTIMICROBIAL THERAPY**	**GENERAL WORKING MECHANISM**
AMINOGLYCOSIDE		
	• Amikacin • Gentamycin • Kanamycin • Neomycin • Netilmicin • Paromomycin • Sisomicin • Streptomycin • Tobramycin	Bactericidal – Inhibits bacterial protein synthesis by binding to the 30S ribosomal subunits and causing cell damage
SULPHONAMIDES & TRIMETHOPRIM		
Trimethoprim	• Trimethoprim	Block the action of bacterial dihydrofolate reductase, inhibiting folate synthesis
Sulphonamides	• Sulfacytine • Sulfadiazine • Sulfadoxine • Sulfamethizole • Sulfapyridine • Sulfisoxazole • Sulphamethoxazole	Bacteriostatic – Arrest cell growth by inhibiting folic acid synthesis
QUINOLONE		

ANTIBACTERIAL CLASS	ANTIMICROBIAL THERAPY	GENERAL WORKING MECHANISM
Fluoroquinolones	• Ciprofloxacin • Levofloxacin • Lomefloxacin • Moxifloxacin • Norfloxacin • Ofloxacin	Bactericidal – Inhibits DNA gyrase, interfering with the reproduction of bacterial DNA
Other	• Nalidixic Acid	Prototype quinolone – bactericidal
ANTIBACTERIAL CLASS	**ANTIMICROBIAL THERAPY**	**GENERAL WORKING MECHANISM**
GLYCOPEPTIDE		
	• Dalbavancin • Fosfomycin • Oritavancin • Ramoplanin • Teicoplanin • Telavancin • Vancomycin	Bactericidal – inhibits the production of bacterial cell wall mucopeptide, resulting in cell lysis and death
DNA SYNTHESIS INHIBITORS		
	• Metronidazole • Clofazimine	DNA synthesis inhibitors
RNA SYNTHESIS INHIBITORS		
	• Rifampicin • Rifabutin • Lipopeptides • Polymyxin A, B, C, D, E	Block ribosomal acceptor sites from accepting transfer RNA

ANNEXURE H: APPLICATION OF STATISTICAL MEASUREMENTS

Objectives	Measurements	Study variables		Statistics		
		Dependent	**Independent**	**Descriptive**	**Inferential**	**Practical significance**
Determine the prevalence of secondary *Candida* infections	Prevalence of infection	Number of patients diagnosed with secondary *Candida* infections		Frequency (%)		
Investigate antibiotic exposure as a risk factor for secondary *Candida* infections	Gender	Secondary *Candida* infection status (Yes/No) Yes – cases group No – control group	Gender (Male or Female)	Frequency (%)	Chi-square test	Cramer's *V*
	Age groups	Secondary *Candida* infection status (Yes/No) Yes – cases group No – control group	Age groups	Frequency (%)	Chi-square test	Cramer's *V*
	Route of administration	Secondary *Candida* infection status (Yes/No) Yes – cases group No – control group	Route of administration (IV or oral treatment)	Frequency (%)	Chi-square test	Cramer's *V*
	Duration of exposure to antibiotic treatment	Secondary *Candida* infection status (Yes/No) Yes – cases group No – control group	Duration of antibiotic treatment (≥ 7 days or <7 days)	Frequency (%)	Chi-square test	Cramer's *V*
	Quantity of antibiotics	Secondary *Candida* infection status (Yes/No) Yes – cases group No – control group	Quantity of antibiotics	Frequency (%)	Chi-square test	Cramer's *V*
Objectives	**Measurements**	**Study variables**	**Statistics**			
		Dependent	**Independent**	**Descriptive**	**Inferential**	**Practical significance**

Objectives	**Measurements**	**Study variables**		**Statistics**		
		Dependent	**Independent**	**Descriptive**	**Inferential**	**Practical significance**
Determine the LOS implication due to secondary *Candida* infection in the ICU	Length of ICU stay stratified by secondary *Candida* infection status	LOS	<u>Main independent variable:</u> Secondary *Candida* infection status (Yes/No) <u>Possible confounders:</u> Age Gender Quantity of antibiotics Route of administrations Duration of exposure to antibiotic treatment	Mean ± standard deviation 95% confidence interval Median interquartile range	Independent *t*-test Poisson regression	Cohen's d
Antibiotic prescribing patterns before diagnosis with secondary C*andida* infections		Number of patients diagnosed with secondary C*andida* infections	Name of active ingredient (Antibiotic)	Frequency (%)		

ANNEXURE I: LANGUAGE EDITING

To whom it may concern

Cecile van Zyl
Language editing and translation
Cell: 072 389 3450
Email: Cecile.vanZyl@nwu.ac.za

28 May 2019

Dear Mr / Ms

Re: Language editing of dissertation (Antibiotic exposure as a risk factor for secondary Candida infections in a private hospital intensive care unit)

I hereby declare that I language edited the above-mentioned dissertation by Ms AM Pieterse (student number: 20321422).

Please feel free to contact me should you have any enquiries.

Kind regards

Cecile van Zyl

Language practitioner

BA (PU for CHE); BA honours (NWU); MA (NWU)
SATI number: 1002391

ANNEXURE J: TECHNICAL EDITING

E Oosthuizen
Technical Editing
Cell: 083 475 5363
E-mail: excellentia.editing@gmail.com

To whom it may concern

28 May 2019

Dear Mr / Ms

RE: TECHNICAL EDITING

I hereby confirm that I have done the technical editing of the dissertation of Ms E Pieterse with the title:

Antibiotic exposure as a risk factor for secondary *Candida* infections in a private hospital intensive care unit

Technical editing includes checking that all styles are applied in the right manner, all captions are inserted correctly and that the table of contents updates.

Kind regards

Engela Oosthuizen