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Safety and efficacy with colistin use: a South African tertiary level experience

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SAFETY AND EFFICACY WITH COLISTIN USE – A SOUTH AFRICAN TERTIARY LEVEL EXPERIENCE

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Medicine.

Johannesburg, 2020

DECLARATION

I, Micky Mammen Sunnyraj, declare that this research report is my own, unaided work. It is submitted (in the submittable format with my protocol and extended literature review) for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.



Signed at Johannesburg on 30/4/2021

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I want like to thank my wife, my family and friends who have always motivated me to complete anything, including this research report.

ABSTRACT

Background:

Clinical experience with colistin, one of the last resort agents against multi-drug resistant gram-negative bacteria (MDR-GNB), shows clinical response varying between 45% to 88%. Two previous South African studies have shown in-hospital mortality rates to vary between 29.6% and 50%. There is also a considerable variation in nephrotoxic potential of colistin which ranges between 20% to 60%. No studies examine the safety and efficacy of colistin in a predominantly HIV infected adult population.

Aim:

To assess the safety and efficacy of colistin in a South African tertiary public health-care facility.

Methods:

We performed a retrospective review of adult patients treated with intravenous colistin at Chris Hani Baragwanath Academic Hospital (CHBAH) between June 2014 to May 2017. Patients were identified through Section 21 applications to the South African Health Products Regulatory Authority (SAHPRA). CHBAH hospital charts and microbiological laboratory records were also available. Demographics, clinical characteristics including features of infection, admitting departments, sites of cultures, the spectrum of organisms cultured, and specifics of therapy with colistin were all recorded. We reported clinical and microbiological outcomes, as well as the incidence of nephrotoxicity.

Results:

Of 118 patients treated (mean age of 42.4 years, with documented HIV positivity in 45 patients (38.1%)), favourable clinical and microbiological outcomes were seen in 70 patients (59.3%) and 71 patienCASts (74.5) respectively. Bacteraemia was noted in 77 patients (65.3%), and *Acinetobacter baumannii* and *Klebsiella pneumoniae* were the commonest organisms isolated from all sources and were cultured from 80 patients (67.8%) and 23 patients (19.5%) respectively. Clinical treatment failure was noted in 11 patients (9.3%), and 14-day-mortality was observed in 37 patients (31.4%). Logistic regression showed that HIV positivity was not associated with adverse outcomes, and male gender was significant for a favourable clinical outcome. Nephrotoxicity was found in 37 patients (31.4%).

Conclusion:

Colistin was effective in the treatment of MDR-GNB in our setting, and HIV positivity was not associated with an adverse outcome.

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ABBREVIATIONS

°C	Degrees Celsius
A. baumannii	*Acinetobacter baumannii*
AIN	Acute interstitial nephritis
AKIN	Acute Kidney Injury Network
APACHE II	Acute Physiology and Chronic Health Evaluation II
ART	Anti-retroviral treatment
AST	Antibiotic susceptibility testing
ATN	Acute tubular necrosis
AWaRe	Access, Watch and Reserve (WHO groups of antibiotics)
BD	Dosed twice-daily
BMD	Broth microdilution
CBS	Colistin base activity
CD_4	Cluster of differentiation 4
CEO	Chief Executive Officer
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	Confidence interval
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CLSI	Clinical and Laboratory Standards Institute
CMS	Colistimethate sodium
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CPE	Carbapenemase-producing *Enterobacteriaceae*
CrCl	Creatinine clearance
CRE	Carbapenem resistant *Enterobacteriaceae*
CRP	C-reactive protein
CVVHD	Chronic veno-venous haemodialysis
DDD	Defined daily dose
e-GFR	Estimated glomerular filtration rate
E. Coli	*Escherichia Coli*
EMAT	Empirical antimicrobial therapy
ESBL	Extended spectrum beta-lactamase producing *Enterobacteriaceae*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GFR	Glomerular filtration rate
GNB	Gram-negative bacteria
HD	Haemodialysis
HIV	Human immunodeficiency virus
HREC	Human Research and Ethics Committee
ICU	Intensive Care Unit
IQR	Inter-quartile range
IT	Intra-thecal
IU	International Units
IV	Intravenous
IVT	Intraventricular
K. pneumoniae	*Klebsiella pneumoniae*
KIDGIO	Kidney Disease Improving Global Outcomes
LPS	Lipopolysaccharide

MCC	Medicines Control Council
MCR	Mobile colistin resistance
MDR	Multi-drug resistant
MDRD	Modification of Diet in Renal Disease
MIC	Minimum inhibitory concentration
MU	Million units
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
OD	Dosed once-daily
OR	Odds-ratio
P. aeruginosa	*Pseudomonas aeruginosa*
PE	Pulmonary embolus
PCT	Procalcitonin
RCTs	Randomised control trials
RIFLE	Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease
RRT	Renal replacement therapy
SAHPRA	South African Health Products Regulatory Authority
SASOCP	South African Society of Clinical Pharmacology
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment score
spp.	Species
TDS	Dosed thrice-daily
WCC	White cell count
WHO	World Health Organisation

CHAPTER 1 – PROTOCOL WITH EXTENDED LITERATURE REVIEW

1. BACKGROUND AND LITERATURE REVIEW

1.1. Introduction

There is a second coming for bacterial pathogens as a significant cause of disease and mortality. The ceaseless battle between man and "bug" has reached new frontiers as limited therapeutic options exist in our armamentarium for the treatment of carbapenem-resistant gram-negative infections [1]. Antimicrobial resistance has reached unprecedented limits best represented by the evolutionary success of gram-negative pathogens such as extended spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL), carbapenem-resistant *Enterobacteriaceae* (CRE), multi-drug resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [2]. The revival of a forgotten antibiotic, colistin, a cornerstone agent in the treatment of infections due to such pathogens, was a necessity [3]. More recently, the discovery of mobile colistin resistance (MCR) genes, signals a new era in antimicrobial resistance, one with significant health burdens, as the estimated lives lost annually to resistant and untreatable infections could amount to 10 million by 2050 [4].

Despite colistin's abandonment by the medical field, it has been pervasively used in agriculture, mainly for its growth promoting effects in animals and prophylaxis or metaphylaxis in livestock [5]. Colistin is so named as it was derived from the soil-borne *Bacillus polymyxa* subspecies *colistinus* [6]. It is also known as Polymyxin E and is one of two clinically useful options from the Polymyxin group of antibiotics (the other being Polymyxin B) discovered in Japan in 1949 [6-8]. Two decades later, its clinical use was abandoned mainly due to issues with nephrotoxicity and the preference for aminoglycosides [6,8-9]. Yet another two decades later, antimicrobial resistance had

forced colistin's return into clinical use, and it is currently one of the "reserve group antibiotics" as defined by the World Health Organisation (WHO) Essential Medicines List Access, Watch and Reserve classification [10-11]. The identification of the transferrable MCR gene, and especially in isolated clinical cases, highlight the need for more stringent regulation and monitoring of antimicrobials used in both animals and humans.

1.2. South African Guidelines for Colistin Use

The principal concern with the resurrection of colistin (which received marketing approval in the 1950s) is that it was never subjected to modern drug development measures and pharmacokinetic analysis that is undergone by any new antimicrobial agent that enters the market in the 21st century [12-13].

Transmissible colistin resistance genes and the drying reservoirs for treatment of resistant gram-negative infections mean that ample measures should be taken to minimise further resistance to colistin. Adequate dosing is essential to this, and two published South African dosing guidelines (see ***Appendix A***) have addressed this issue [12,14]. Additionally, these guidelines assist healthcare workers in the optimal treatment of multi-drug resistant Gram-negative bacteria (MDR-GNB) by striving to provide ideal therapeutic dosing, all the while reducing toxicity. The South African Society of Clinical Pharmacy (SASOCP) guideline employs creatinine clearance (CrCl) using the Cockcroft-Gault formula to evaluate the glomerular filtration rate as opposed to estimated glomerular filtration rate (e-GFR) using either Modification of Diet in Renal Disease (MDRD) or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas. Although the MDRD or the newer CKD-EPI formula produces more accurate estimates of GFR in comparison to CrCl estimates using the

much older Cockcroft-Gault equation, using CrCl remains a viable strategy for dose adjustments in renal failure as most drugs are validated using this method.

Some colistin dosing regimens used in clinical practice result in sub-therapeutic colistin plasma concentrations leading to the selection of resistant bacteria with higher minimum inhibitory concentrations (MICs) [12,15]. In vitro studies into colistin pharmacodynamics and combination therapy with specific antimicrobial agents suggest that the use of an 8-hourly dosing regimen and combination therapy is associated with a lower likelihood of the emergence of resistance [16]. The South African Society of Clinical Pharmacy guideline also puts weight on an 8-hourly maintenance regimen in adult patients with normal renal function, and both dosing guidelines suggest that combination therapy maximises antimicrobial activity and reduces resistance [12,14]. A recent South African study, however, showed significantly shorter treatment durations in patients who received higher loading doses as well as maintenance doses of 4.5 million units (MU) intravenously (IV) 12-hourly [17].

1.3. Colistin in South Africa

Colistin serves as an example of how many South African stakeholders have engaged in an attempt to alleviate a public health concern, that of transmissible colistin resistance; through advocating for regulatory changes, increased surveillance and broadening antimicrobial stewardship throughout the various sectors which use this agent [5].

Parenteral colistin is not registered for use in humans in South Africa; however, Section 21 of Act 101 of 1965 allows the South African Health Products Regulatory Authority (SAHPRA) [previously known as the Medicines Control Council] to provide permission to institutions to import the product under another jurisdiction [12-13]. This

administrative challenge in accessing one of the last line therapeutic agents required to treat carbapenem-resistant gram-negative infections may be seen as a matter of concern, as firstly, it could prove to be obstructive in the timely initiation of a life-saving antibiotic, and secondly, such severe infections are quite common to critical care settings in South Africa [13,18]. However, such stringent regulatory processes are a vital part of antimicrobial stewardship in preventing disseminated use and the development of antimicrobial resistance to one of the last line antibiotics. South Africa imports a European colistimethate sodium (CMS) product for parenteral use and hence uses the European dosing convention (see ***Appendix B***) [6,12].

In stark contrast, colistin is registered for use in animals in South Africa, allowing its use as a growth promoting agent as well as its veterinary use in livestock. As an illustration to the magnitude of colistin used in this sector, one report mentions that 4 tonnes of colistin sulphate powder was sold in 2015 alone. This excludes colistin in the parenteral formulation as well as compounded colistin-based medicines for used for veterinary purposes [5]. This emphasises the importance of stewardship of colistin at a national and an international level as mitigating transmissible colistin resistance is a task beyond the scope of clinical medicine alone and requires changes in national policies and regulations in other sectors outside of medicine.

1.4. Mechanism of Action

Despite it being one of the golden-age antibiotics and after six decades of clinical use, the exact mechanism by which colistin brings about its antimicrobial effect is not fully understood [19-21]. Colistin disrupts the cell membranes of gram-negative bacteria through electrostatic interaction with phospholipids and competitive displacement of divalent cations such as calcium and magnesium [6, 22-23]. Many experts believe in

exploiting this mechanism of its action by using combination therapy to maximise the antimicrobial effect of other antibiotics (such as carbapenems) due to colistin's detergent activity on the gram-negative bacterial cell wall [6,12,14]. Colistin is also thought to have an anti-endotoxin effect by binding to and neutralising the Lipid A part of the LPS molecule [23].

1.5. Spectrum of Action

Notwithstanding its narrow spectrum of action, colistin's utility is that exerts an antibacterial effect on select resistant gram-negative organisms (extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae* (CPE), MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* responsible for nosocomial infections, especially in critical care settings [2,6,9,12]. Specific organisms are intrinsically resistant to colistin; some examples include the *Proteus* spp., *Morganella morganii*, *Providencia* spp., *Serratia marcescens*, *Burkholderia cepacia*, *Brucella, Legionella*, *Campylobacter*, and *Vibrio cholerae* [23]. Furthermore, colistin does not have activity against gram-positive bacteria, anaerobic organisms and gram-negative cocci.

1.6. Modes and Mechanisms of Resistance to Colistin

Resistance to colistin was once thought to be rare, acquired slowly and chromosomally. Recently, there has been global concern after the discovery of plasmid-mediated colistin resistance that can be transmitted horizontally, implying the potential for widespread dissemination of colistin resistance across many bacterial species [5]. The mechanisms by which GNB acquire colistin resistance are complex and have not been fully elucidated; however, it is thought to be primarily through

modification of LPS or more specifically the Lipid A molecule's structure or charge and less commonly by the termination of LPS production, outer membrane porin modification and the activation of efflux pumps [6,19]. Plasmid-mediated colistin resistance was initially described in China in species of *E. coli* and *K. pneumoniae* during the periods 2011 to 2014, and it is hypothesised to have evolved from selective pressures exerted by colistin used extensively in the agriculture industry. To date, nine such plasmid-mediated MCR genes have been identified, and the MCR-1 gene has been described on every continent and isolated from both animal and human populations [19]. The SENTRY antimicrobial surveillance programme provided evidence to suggest that resistance to colistin is increasingly reported in clinical isolates (especially in *Klebsiella Pneumoniae* species) both world-wide and in South Africa [18-19]. ResistanceMap, an open-access reference website reporting antibiotic consumption and resistance data, showed that resistance to colistin was seen in 1% of *Klebsiella pneumoniae* and in 2% of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clinical isolates tested in South Africa in 2016 [24]. Highlighting the looming apocalypse is the recent identification of MCR-1 genes isolated in highly resistant *Enterobacteriaceae* also containing plasmid-mediating carbapenemases indicating that we are indeed capable of manufacturing nosocomial superbugs resistant to all antibiotics [20].

1.7. Routes of Administration, Colistin Formulations and Dosing Considerations

Over the past decade, a litany of research into the pharmacodynamics and pharmacokinetics of colistin has attempted to explore its secrets and resolve the scientific shortcomings of the 1950s. Despite this, there are reports that much of the prescribing information supplied with the product still contains non-standardised dosing instructions and outdated pharmacokinetic data due to invalid microbiological assays used before marketing approval obtained decades ago [13,15].

1.7.1 Routes of Administration

Polymyxins are not absorbed by the gastrointestinal tract and their distribution in most organs is poor; furthermore, it does not cross the blood-brain barrier in non-inflamed meninges [6,23]. Colistin could, however, be used through the intra-thecal route or intraventricular administration for MDR-GNB infections of the central nervous system (CNS) [6]. Numerous studies have observed that inhaled colistin has been effectively used in the treatment of *P. aeruginosa* in patients with cystic fibrosis [23]. The issue is that data regarding the use of inhaled colistin in the management of GNB pneumonias is sparse, and multiple randomised controlled trials (RCTs) have shown a lack of benefit as well [6]. There are concerns with using aerosolised colistin including that it may allow resistance to colistin in MDR-GNBs and that the drug breakdown products are toxic to lung tissue, significantly when the preparations are diluted for periods of greater than 24 hours before use [6,23].

1.7.2 Colistin Formulations

Colistin is currently only available in forms that are both non-absorbable from the gastrointestinal tract, namely colistimethate sodium (CMS) and colistin sulphate [6]. Colistin sulphate is used only for topical administration and for non-absorbable oral

use [6]. Colistimethate sodium (CMS) is also known as colistimethate sulphonate (also abbreviated CMS) [6,12-14]. CMS is the parenteral pro-drug that is hydrolysed after administration to produce numerous by-products, only one of which is the active drug colistin [6,12-13]. There is much confusion caused by the nomenclature as well as the separate dosing conventions used for two different internationally available formulations of CMS (see ***Appendix B***) [6,12-13].

1.7.3 Dosing Considerations

The half-life of colistin is 14.4 hours, and colistin requires about 4-5 half-lives to reach a steady-state [12]. Hence the purpose of a loading dose is used to achieve therapeutic concentrations rapidly. Plasma concentrations peak 7 hours after administration, and as mentioned above, its distribution into other compartments (such as CSF, pleural and synovium) is poor [6,12]. It is important to note that due to capillary leakage in critically-ill patients, there is an increase in the volume of distribution of about 4 to 15-fold and hence, these patients require a higher loading dose to reach a therapeutic level [12,25]. In patients with renal dysfunction, the bioavailability of colistin is increased as the pro-drug (CMS) is less readily excreted and is increasingly converted to colistin, and hence renal adjustment is necessary to mitigate nephrotoxicity. In patients on dialysis with either haemodialysis (HD) or veno-venous hemofiltration, a post-dialysis dose of colistin is required as both the pro-drug (CMS) and colistin are cleared during dialysis [12,26-27].

1.8. Polymyxin E (Colistin) Versus Polymyxin B (PMB)

An alternative to colistin may be PMB, especially in critically ill patients at risk for nephrotoxicity, since the conversion of the pro-drug (CMS) to colistin is often variable and leads to unpredictable serum concentrations (see ***1.7.3 Dosing Considerations***). Despite being almost identical chemical structures and having similar mechanisms of action, resistance patterns and spectrum of activity, PMB and colistin differ greatly in their pharmacodynamic and pharmacokinetic properties [6]. The advantage of PMB is its ability to rapidly provide predictable serum concentrations without the need for either conversion into an active form, or renal adjustments and at a lower cost [28, 29]. PMB is also associated with lower rates of nephrotoxicity and is eliminated via non-renal mechanisms [28]. Despite this, efficacy of colistin is equal to that PMB and studies have shown that there is no difference in mortality [30]. Much more research into the pharmacodynamics of colistin, bioavailability and published guidelines for its dosing has made it a more favourable option than its comparable counterpart.

1.9. Colistin Monotherapy Versus Combination Therapy

Yet another debate that has emerged since the revolution of treatment of MDR-GNB with colistin is whether it should be used as monotherapy or in combination with other agents. Colistin is commonly used as part of combination therapy with carbapenems in many settings for the management of MDR-GNB infections, but the evidence for this is mainly based on the results of observational studies [31]. Given colistin's mechanism of action (see ***1.4. Mechanisms of Action***) and that specific agents are effective in-vitro such as azithromycin, tigecycline, carbapenems, rifampicin and doxycycline, combination therapy for synergistic antimicrobial effect seems a reasonable choice [23]. A recent systematic review and meta-analysis looking at both

RCTs and observational studies showed that combination therapy with colistin was superior to monotherapy and reinforced this practice [32]. Prominent arguments advocating against the use of combination therapy include higher costs of treatment, increased risk of *Clostridioides difficile* and fungal infections but primarily, increased risk of adverse effects such as nephrotoxicity [32]. Navigating antimicrobial resistance is a double-edged sword, especially in settings of poor antimicrobial stewardship, as the increased use of antimicrobials in combination promotes a general increase in selection pressure and hence the development of resistance, but the use of colistin monotherapy promotes increased resistance to colistin [32].

Furthermore, higher rates of survival with combination treatments in observational studies are not often replicated in RCTs where there is often little difference between colistin monotherapy or combination therapy for the management of patients with MDR-GNB infections [33]. This was emphasised by a systematic review and meta-analysis of 32 studies published studies up to November 2016 which suggested that the use of intravenous colistin combination therapy was not associated with lower mortality when compared to intravenous colistin monotherapy [33]. The same meta-analysis did, however, mention that combination therapy could be efficacious in the treatment of patients with bacteraemia and *Acinetobacter* infections as there was a statistically significant outcome in favour of intravenous combination therapy in both these groups [33]. Another recent meta-analysis examining five RCTs that included 791 patients found no differences between colistin monotherapy and colistin based combination therapy against carbapenem-resistant GNB infections and especially in the case of *A baumannii* infections [34]. This meta-analysis looked at four antibiotics that were used as part of combination therapy with colistin, and this included rifampicin, fosfomycin, sulbactam and meropenem. Unfortunately, more well-designed

RCTs are required to end the debate of colistin monotherapy versus combination therapy ultimately.

1.10. Clinical Response

Randomised controlled trials are still lacking to shed light on its efficacy and safety as the treatment of critically ill patients with colistin poses many challenges. A recent systematic review and meta-analysis of 4 RCTs and 14 observational studies that evaluated the use of colistin as therapy for *A baumannii* infections showed that colistin monotherapy was effective in the management of such infections and that when used in combination with other antibiotics, the risk of nephrotoxicity was lower [35]. A meta-analysis of 9 RCTs of 940 patients focused on evaluating the safety and efficacy of colistin in patients with pulmonary infections caused by either *Pseudomonas aeruginosa,* or *Acinetobacter baumannii* showed no difference concerning efficacy, morality and nephrotoxicity associated with colistin use when compared to standard antibiotics [23].

The clinical response to colistin has been reported to be between 45% and 88% [36-37]. Mortality rates and clinical outcomes in the critically-ill with MDR-GNB infections are dependent on a multitude of factors aside from the choice of antibiotic and most often patient factors such as admission to ICU and high APACHE scores which influence adverse outcomes [36-39].

Locally available data regarding the efficacy of colistin is limited. One South African study compared the use of colistin to tobramycin in the treatment of resistant *A baumannii* infections in an ICU [36]. The study looked at factors such as ICU survival, in-hospital mortality, time to microbiological clearance and elevations in serum creatinine [35]. It showed that both colistin and tobramycin were equally efficacious and

produced similar outcomes concerning nephrotoxicity [35]. Both the in-hospital mortality and bacteriological eradication for patients treated with colistin was 50% in this study [35]. Another study looking at the use of colistin and carried out in three private sector facilities showed an in-hospital mortality rate of 29.6% [17]. International mortality outcomes from several observational studies vary widely between 10 – 70% [8,23,37-38]

1.11. Adverse Events

There are several documented adverse reactions to colistin which include nephrotoxicity, neurotoxicity and hypersensitivity reactions; the commonest of which is nephrotoxicity [6].

There is significant variation in data regarding the prevalence of nephrotoxicity in patients treated with colistin before its resurgence and more recently. This can be attributed to several factors, including the use of CMS rather than colistin sulphate, improved purification methods, dosing adjustments in renal dysfunction and increased awareness for other causes of nephrotoxicity and renal dysfunction [23].

CMS is eliminated by tubular excretion, and the mechanism of renal elimination of colistin is poorly understood [23]. Eighty per cent of colistin is reabsorbed in the renal tubules; hence most of the filtered colistin is recovered. This suggests that non-renal mechanisms clear colistin [26]. The mechanisms by which colistin causes nephrotoxicity is poorly understood but seem to be multifactorial. In-vitro electrophysiological studies in mammalian tissues have revealed that prolonged exposure with colistin increases trans-epithelial conduction of urothelium and this seems to suggest that the mechanism of toxicity of tubular epithelium is similar to its action on bacterial cell membranes, increasing tubular epithelial membrane permeability and hence increasing the influx of water and ions eventually resulting in

cell swelling and lysis [21,26]. Thus, the main mode of nephrotoxicity is acute tubular necrosis (ATN) as evidenced by elevations in serum urea and creatinine levels. There is also some evidence to suggest that colistin nephropathy is associated with oxidative stress and caspase-associated apoptosis [23]. Furthermore, there have also been a few case reports of acute interstitial nephritis (AIN) as a result of hypersensitivity reactions to polymyxins [26].

The nephrotoxic potential of colistin is dependent on both the concentration as well as the length of exposure but is known to be reversible [23,26]. Many constraints preclude effectively assessing colistin associated nephrotoxicity in observational studies including factors such as the frequently encountered renal dysfunction in sepsis, the concomitant use of nephrotoxic agents, the severity of patient illness and co-morbid conditions. Consequentially, risk factors for nephrotoxicity with colistin use aside from the dose and duration of therapy include the use of concomitant nephrotoxins as well as patient-related factors such as age, sex, hypoalbuminemia, hyperbilirubinemia, co-morbid conditions and the severity of the patient's illness [26-27].

The incidence of nephrotoxicity due to colistin in South Africa is not known, however, as mentioned above, the reversible nephrotoxic potential of colistin is dose and duration dependent and varies widely from 20 to 60 per cent [6]. One of the first prospective studies that confirmed this reversible dose-dependent toxicity potential of colistin also showed the incidence of nephrotoxicity with colistin to be 14% [27]. Some of this discrepancy is due to variable definitions of nephrotoxicity adopted by researchers over the years as well as lack of a common dosing scheme [40].

The association between neurotoxic effects and colistin use has come into question as more recent data suggest that this association is weak. Additionally, it is possible that many confounding factors exist in the retrospective cohort studies that have

reported it [23]. The mechanisms of neurotoxicity associated with many of colistin's neurological symptoms are not well understood [6]. Furthermore, colistin has been associated with apnoea and respiratory failure as a result of a non-competitive neuromuscular blockade that is not responsive to neostigmine [6].

The neurotoxic potential of colistin is more prevalent in patients with cystic fibrosis. Reported acute neurotoxicity was more prevalent in women, and the incidence of neurotoxicity was not affected by age [6].

To our knowledge, there is no local data on the incidence of neurotoxicity associated with colistin use. There is also reporting of other toxic effects of colistin use, including hypersensitivity reactions and gastrointestinal symptoms, but the association of colistin use with these symptoms are inconclusive and data is sparse.

Many factors limit the use of colistin in South Africa, one of our last line agents in the management of carbapenem resistant gram-negative infections. Although guidelines now exist for rational prescribing, there is no international consensus regarding many aspects of therapy, including ideal dose and use of combination therapy.[6,10-13,15,25] In a country where it is not even registered for use; data regarding our experience with colistin, especially regarding its safety and efficacy as a therapeutic agent, is scanty.[6,12-13,39] This research project aims to share our experience at a tertiary level facility and to evaluate the use of colistin vis-à-vis safety and efficacy.

2. RESEARCH QUESTION

Is the South African experience of colistin use in adults with carbapenem-resistant infections comparable to existing safety and efficacy data?

3. OBJECTIVES

1. To determine the efficacy of colistin by means of:
    1.1. Measuring 14-day mortality
    1.2. Clinical responses to colistin
    1.3. Microbiological/bacteriological responses to colistin
2. To assess the safety of colistin use by means of:
    2.1. Establishing the rates of toxicity associated with colistin use
3. To evaluate the microbiological spectrum of organisms treated with colistin

4. METHODS

4.1. Study design, Study Population and Sample

The proposed study is a retrospective cohort study of adult patients (≥18 years of age) admitted to Chris Hani Baragwanath Academic Hospital (CHBAH), between June 2014 to May 2017, who were treated with intravenous colistin for a minimum of 72 hours for microbiologically documented evidence of a carbapenem-resistant gram-negative infection.

Convenience sampling will be used to identify patients employing CHBAH pharmacy records of Section 21 applications to the MCC for colistin use. These records will then be correlated with CHBAH hospital charts and microbiological laboratory records regarding demographical, clinical and microbiological data, including the identification and antibiotic sensitivities of the organisms involved.

The sites from which the carbapenem-resistant organisms are cultured will be documented and will be classified into the following: bloodstream infection, sputum/tracheal aspirate, wound pus swab, pus, cerebrospinal fluid, central venous catheter tip, urine and other (including positive cultures from non-specified sites).

Colistin use is seen as acceptable in patients who have had a positive microbiological culture for a gram-negative organism sensitive to colistin and also have met one or more of the following criteria or clinical signs to suggest infection (temperature $\geq$ 38°C, an elevated C-reactive protein (CRP), an elevated procalcitonin (PCT), a white cell count $\geq$ $12x10^9$/L or $\leq$ $4x10^9$/L, a heart rate of greater than 90 beats per minute, presence of purulent sputum/tracheal aspirate, a respiratory rate greater than 20 or necessity for ionotropic support) [37].

4.2. Endpoints

4.2.1. Efficacy of colistin use will be determined by measuring the endpoints of 14-day mortality, clinical and bacteriological clearance.

There is evidence to suggest that thirty days is too long to interpret the cause of mortality in critically ill patients and seven days is too short to evaluate outcome hence, all-cause mortality will be evaluated at day fourteen after initiation of treatment of colistin [41].

Clinical clearance will be evaluated based on subjective clinical assessments recorded by the attending physician at the end of treatment with colistin as being *favourable, unfavourable* or *indeterminate* [37].

A *favourable outcome* is a composite indicator that would include complete or partial resolution of signs and symptoms and improvement

of inflammatory biomarkers (white cell count, C reactive protein and procalcitonin) without antibiotic maintenance therapy [42].

Unfavourable outcomes would encompass cases of treatment failure and all-cause 14-day mortality.

Treatment failure is a composite that refers to the persistence of signs and symptoms and persistently elevated inflammatory biomarkers or reoccurrence of signs and symptoms after discontinuation of colistin [37,42].

All-cause 14-day mortality after initiation of treatment would also be used as a variable in the assessment of an unfavourable outcome.

An *indeterminate* outcome would be defined as the inability to assess clinical clearance [37].

Microbiological clearance will be determined by means of examining follow-up cultures of patients that received colistin treatment. These will subsequently be evaluated as *eradication*, *treatment failure* or *super-infection* [37].

Eradication is defined as bacteriological clearance of the same organism from the same site on a follow-up culture, wherever available [37].

Cases, where there is the persistence of the organism on follow-up microbiological culture, would be termed as *treatment failur*e [37].

Situations, where different organisms are isolated from follow up cultures from the same sites, would be termed *super-infection* [37].

4.2.2. Safety of colistin use will be evaluated by measuring the endpoint of toxicity rates associated with colistin use. Expected side effects based on the literature review include nephrotoxicity and neurotoxicity.

In patients with normal renal function at baseline, nephrotoxicity will be defined as a greater than two-fold increase in serum creatinine from baseline value anytime during colistin treatment [37]. The attending physician's notes will be evaluated for documented reductions in urine output.

In patients with pre-existing renal failure, nephrotoxicity will be defined as greater than 50% increase in the serum creatinine level from baseline value or a decline in renal function that required renal replacement therapy (RRT) [37].

The attending physician's notes will be evaluated for evidence of documented neurotoxic events such as neuromuscular blockade, seizures, ataxia, diplopia and paraesthesia [6,38-39].

4.3. Microbiology

All cultures and antibiotic susceptibility testing at CHBAH are done by the National Health Laboratory Service (NHLS) at the hospital. Standard screening methods are used by the laboratory to identify gram-negative organisms isolated from routine cultures. Antibiotic susceptibility testing (AST) for all other antibiotics is performed by the MicroScan Walkaway (Siemens, New York, USA)© automated system according to manufacturer instructions. A 2016 joint European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) subcommittee recommended broth microdilution (BMD) as the only reliable method to determine colistin susceptibility. Colistin susceptibility testing is done by the Sensititre (Trek, UK)© automated system and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Furthermore, genotypic testing is available from the National Institute of Communicable Diseases (NICD) helped to provide molecular confirmation.

5. DATA ANALYSIS

Data about patient demographics, co-morbidities, hospitalisation, clinical condition, sites of microbiological culture, clinical clearance, microbiological clearance, toxicities and mortality will be reproduced from the datasheet (see ***Appendix D***). This will be captured onto Microsoft Excel (Microsoft Corp, USA) for primary analysis purposes. The Shapiro–Wilks W test will initially be used to test data for normalcy. Demographic and clinical characteristics will be expressed as percentages for categorical variables (e.g., gender, co-morbid conditions, pathogens isolated, clinical clearance, microbiological clearance); means and medians will be used to express continuous variables (e.g., age, APACHE II scores, length of hospital stay and duration of colistin use) and will be supplemented by their standard deviations and interquartile ranges respectively. Furthermore, for normally distributed continuous variables, t-test or the ANOVA test will be used to analyse the significance of associations. For non-normally distributed continuous variables, analyses can be made using non-parametric Mann–Whitney U tests. Chi-square ($\chi 2$) tests will be used to analyse any categorical data. P-value < 0.05 will denote significance for all statistical analyses. SPSS Statistics software (SPSS Inc., Chicago, IL, USA) will be used to perform univariate and multivariate data analysis. A univariate analysis will be performed to assess factors associated with clinical outcomes and mortality. All plausible significant variables from a univariate analysis will be analysed in a multivariate analysis. We may be able to use Kaplan- Meier survival analysis to determine the time to mortality (which is defined as the interval between initiation of treatment with colistin and death) in HIV infected and HIV non-infected as a possible secondary outcome for the study.

6. ETHICAL CONSIDERATIONS

During the data collection process, various measures will be set in place to ensure participant anonymity. Approval to conduct the study at CHBAH will be obtained from the Chief Executive Officer (CEO) of the hospital as well as from the Head of Department of Pharmacy at CHBAH. Ethics approval for the study will be obtained from the Human Research and Ethics Committee (HREC), University of the Witwatersrand.

7. LIMITATIONS

We envisage that this study will have some limitations.

1. The study may rely on an incomplete database of participants who have received colistin at CHBAH due to issues such as failure to complete Section 21 of the MCC application forms, incorrectly completed application forms and misplaced records.
2. This is a proposed retrospective study that will use convenience sampling, and this may thus limit the generalisability of results. The data recorded and its quality may contribute to bias. Patients may also have died prior to receiving microbiological results and others may have died prior to receiving colistin.
3. Treatment of culture positive patients with colistin was based on clinical judgement of the attending physician and it is possible that some of the patients may have been colonised with the organism or that the organism cultured was a contaminant. This may confound results.
4. It is difficult to truly assess effectiveness of colistin without comparison of therapy to a control group.

5. The use of combination therapy with colistin will limit our conclusions regarding clinical and microbiological effectiveness.
6. Numerous confounding variables may also exist for mortality and toxicities associated with colistin use.

8. FUNDING

No external funding is required for the completion of this study.

9. REFERENCES

1 Sood S. Chloramphenicol - A Potent Armament Against Multi-Drug Resistant (MDR) Gram Negative Bacilli? J Clin Diagn Res. 2016;10(2):DC01-03.

2 Kanj SS, Kanafani ZA. Current Concepts in Antimicrobial Therapy Against Resistant Gram-Negative Organisms: Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae, Carbapenem-Resistant Enterobacteriaceae, and Multidrug-Resistant Pseudomonas aeruginosa. Mayo Clin Proc. 2011; 86(3):250-259.

3 Sarkar S, Evelyn R, DeSantis H, Kuper J. Resurgence of colistin use. Am J Health-syst Pharm. 2007;64:2462-6.

4 O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Analysis and Policy Observatory [Internet]. 2016 [cited 11/1/2020].
Available from: https://apo.org.au/node/63983

5 Mendelson M, Brink A, Gouws J, Mbelle N, Naidoo V, Pople T et al. The One Health Stewardship of Colistin as an Antibiotic of Last Resort. Lancet Infect Dis [Internet]. 2018 [cited 11/1/2020].
Available from: http://dx.doi.org/10.1016/S1473-3099(18)30119-1

6 Polymyxins: An overview. Wolters Kluwer [Internet]. 2020 [cited 1/2/2020].
Available from: https://www.uptodate.com/contents/polymyxins-an-overview

7 Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005;40(9):1333-41.

8 Michalopoulos A, Falagas ME. Colistin and polymyxin B in critical care. Crit Care Clin. 2008;24(2):377-91.

9 Overview of carbepenemase-producing gram-negative bacilli. Wolters Kluwer [Internet]. 2020 [cited 1/2/2020].
Available from: https://www.uptodate.com/contents/overview-of-carbapenemase-producing-gram-negative-bacilli

10 Laxminarayan R, Van Boeckel T, Frost I, Kariuki S, Khan EA, Limmathurotsakul D, et al. The Lancet Infectious Diseases Commission on antimicrobial resistance: 6 years later. Lancet Infect Dis. 2020;20(4):e51-e60.

11 Executive summary: the selection and use of essential medicines. Report of the 21st WHO Expert Committee on the Selection and Use of Essential Medicines. WHO [Internet]. 2017 [cited 20/3/2020].

Available from: https://apps.who.int/iris/bitstream/handle/10665/273826/EML-20-eng.pdf?ua=1

12 Kift EV, Maartens G, Bamford C. Systematic review of the evidence for rational dosing of colistin. S Afr Med J. 2014;104(3):183-6.

13 Nation RL, Li J, Cars O, Couet W, Dudley MN, Kaye KS, et al. Framework for optimisation of the clinical use of colistin and polymyxin B: the Prato polymyxin consensus. Lancet Infect Dis. 2015;15(2):225-34.

14 Labuschagne Q, Schellacka N, Gousa A, Bronkhorsta E, Schellacka G, Tondera L et al. COLISTIN: adult and paediatric guideline for South Africa. South Afr J Infect Dis. 2016;31(1):3–7.

15 Landersdorfer CB, Nation RL. Colistin: how should it be dosed for the critically ill? Semin Respir Crit Care Med. 2015;36(1):126-35.

16 Bergen PJ, Li J, Nation RL, Turnidge JD, Coulthard K, Milne RW. Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with Pseudomonas aeruginosa in an in vitro pharmacodynamic model. JAC. 2008;61(3):636-642.

17 Messina AP, Brink AJ, Richards GA, van Vuuren S. Opportunities to optimize colistin stewardship in hospitalized patients in South Africa: Results of a multisite utilization audit. S Afr Med J. 2018;108(1):28-32.

18 Osei Sekyere J. Current State of Resistance to Antibiotics of Last-Resort in South Africa: A Review from a Public Health Perspective. Front Public Health. 2016;4:209.

19 Poirel L, Jayol A, Nordmann P. Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clin Microbiol Rev. 2017;30(2):557-596.

20 Biswas S, Brunel JM, Dubus JC, Reynaud-Gaubert M, Rolain JM. Colistin: An Update on the Antibiotic of the 21st Century. Expert Rev Anti Infect Ther. 2012;10(8):917-934.

21 Nation RL, Li J. Colistin in the 21st Century. Curr Opin Infect Dis. 2009;22(6):535–543.

22 Bennet JE, Dolin R, Blaser MJ. Mandell, Douglas and Bennet's Principles and Practice of Infectious Diseases. 9th Ed. Philadelphia: Elsevier, 2020.

23 Al Ramahi JW, Al Bashaireh A, Salman S. Colistin in Critically Ill Patients: A Critical Review. IAJAA [Internet]. 2014 [cited 11/1/2020];4(2). Available from: http://imed.pub/ojs/index.php/IAJAA/article/view/939

24 ResistanceMap: Antibiotic resistance. The Centre for Disease Dynamics Economics & Policy. [Internet]. 2018 [cited 20/3/2020]. Available from: https://resistancemap.cddep.org/AntibioticResistance.php

25 Nation RL, Garonzik SM, Thamlikitkul V, Giamarellos-Bourboulis EJ, Forrest A, Paterson DL, et al. Dosing Guidance for Intravenous Colistin in Critically Ill Patients. Clin Infec Dis. 2016. 2017;64(5):565-571.

26 Javan AO, Shokouhi S, Sahraei Z. A Review on Colistin Nephrotoxicity. Eur J Clin Pharmacol. 2015;71(7):801-810.

27 Falagas ME, Fragoulis KN, Kasiakou SK, Sermaidis GJ, Michalopoulos A. Nephrotoxicity of intravenous colistin: a prospective evaluation. Int J Antimicrob Agents. 2005;26(6):504-507.

28 Cai Y, Lee W & Kwa AL. Polymyxin B versus colistin: an update. Expert Rev. Anti-infect. Ther. 2015;13:12:1481-1497.

29 Colistin vs Polymyxin B. Relias Media. 1/7/2016. [cited 12/11/2020]. Available from: https://www.reliasmedia.com/articles/138130-colistin-vs-polymyxin-b

30 Vardakas KZ, Falagas ME. Colistin versus polymyxin B for the treatment of patients with multidrug-resistant Gram-negative infections: a systematic review and meta-analysis. Int J Antimicrob Agents. 2017;49(2):233-238.

31 Bassetti M, Peghin M, Vena A and Giacobbe DR. Treatment of Infections Due to MDR Gram-Negative Bacteria. Front Med (Lausanne). 2019;6:74.

32 Schmid A, Wolfensberger A, Nemeth J, Schreiber PW, Sax H, Kuster SP. Monotherapy versus combination therapy for multidrug-resistant Gram-negative infections: Systematic Review and Meta-Analysis. Sci Rep. 2019;9: 15290.

33 Vardakas KZ, Mavroudis AD, Georgiou M, Falagas ME. Intravenous colistin combination antimicrobial treatment vs. monotherapy: a systematic review and meta-analysis. Int J Antimicrob Agents. 2018;51(4):535–547.

34 Cheng IL, Chen YH, Lai CC, Tang HJ. Intravenous Colistin Monotherapy versus Combination Therapy against Carbapenem-Resistant Gram-Negative Bacteria Infections: Meta-Analysis of Randomized Controlled Trials. J. Clin. Med. 2018;7:208.

35 Wang J, Niu H, Wang R, Cai Y. Safety and efficacy of colistin alone or in combination in adults with Acinetobacter baumannii infection: A systematic review and meta-analysis. Int J Antimicrob Agents. 2019;53(4):383-400.

36 Gounden R, Bramford C, van Zyl-Smit R, Cohen K, Maartens G. Safety and effectiveness of colistin compared with tobramycin for multi-drug resistant Acinetobacter baumannii infections. BMC Infect Dis. 2009;9:26.

37 Desai K, Kazi M, Ajbani K, Munshi M, Rodriges C, Soman R, Shetty A. Clinical outcomes and safety of colistin in treatment of gram-negative infections: A prospective observational study. Egypt J Crit Care Med. 2016;4(2):67-72.

38 Cheng C, Sheng W, Wang J, Chen C, Chang S. Safety and efficacy of intravenous colistin (colistin methanesulphonate) for severe multidrug-resistant Gram-negative bacterial infections. Int J Antimicrob Agents. 2010;35:297-300.

39 Dimitriades K, Morrow BM, Jeena P. A retrospective study on the effects of colistin therapy in children with multidrug-resistant Gram-negative bacterial pathogens: impact of HIV status on outcome. Arch Dis Child. 2014;99(3):262-266.

40 Ozel AS, Ergönül Ö, Korten V. Colistin nephrotoxicity in critically ill patients after implementation of a new dosing strategy. J Infect Dev Ctries. 2019;13(10):877-885.

41 Gu Z, Han Y, Meng T, Zhao S, Gao C, Huang W. Risk Factors and Clinical Outcomes for Patients with Acinetobacter baumannii Bacteremia. Medicine (Baltimore). 2016;95(9):e2943.

42 Cho YS, Yim H, Yang HT, Hur J, Chun W, Kim JH, Lee BC, Seo DK, Kim D. Use of parenteral colistin for the treatment of multi-resistant Gram-negative organisms in major burn patients in South Korea. Infection. 2012;40(1):27-33

CHAPTER 2 – SUBMISSIBLE ARTICLE

Title: Safety and Efficacy with Colistin Use – A South African Tertiary Level Experience

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Conflicts of Interest: Nil

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ABSTRACT

Background:

Clinical experience with colistin, one of the last resort agents against multi-drug resistant gram-negative bacteria (MDR-GNB), shows clinical response varying between 45% to 88%. Two previous South African studies have shown in-hospital mortality rates to vary between 29.6% and 50%. There is also a considerable variation in nephrotoxic potential of colistin which ranges between 20% to 60%. No studies examine the safety and efficacy of colistin in a predominantly HIV infected adult population.

Aim:

To assess the safety and efficacy of colistin in a South African tertiary public health-care facility.

Methods:

We performed a retrospective review of adult patients treated with intravenous colistin at Chris Hani Baragwanath Academic Hospital (CHBAH) between June 2014 to May 2017. Patients were identified through Section 21 applications to the South African Health Products Regulatory Authority (SAHPRA). CHBAH hospital charts and microbiological laboratory records were also available. Demographics, clinical characteristics including features of infection, admitting departments, sites of cultures, the spectrum of organisms cultured, and specifics of therapy with colistin were all recorded. We reported clinical and microbiological outcomes, as well as the incidence of nephrotoxicity.

Results:

Of 118 patients treated (mean age of 42.4 years, with documented HIV positivity in 45 patients (38.1%)), favourable clinical and microbiological outcomes were seen in 70 patients (59.3%) and 71 patients (74.5) respectively. Bacteraemia was noted in 77 patients (65.3%), and *Acinetobacter baumannii* and *Klebsiella pneumoniae* were the commonest organisms isolated from all sources and were cultured from 80 patients (67.8%) and 23 patients (19.5%) respectively. Clinical treatment failure was noted in 11 patients (9.3%), and 14-day-mortality was observed in 37 patients (31.4%). Logistic regression showed that HIV positivity was not associated with adverse outcomes, and male gender was significant for a favourable clinical outcome. Nephrotoxicity was found in 37 patients (31.4%).

Conclusion:

Colistin was effective in the treatment of MDR-GNB in our setting, and HIV positivity was not associated with an adverse outcome.

INTRODUCTION

There is a second coming for bacterial pathogens as a significant cause of disease and mortality. Antimicrobial resistance has reached unprecedented limits best represented by the evolutionary success of gram-negative pathogens such as extended spectrum beta-lactamase-producing (ESBL) *Enterobacteriaceae*, carbapenem-resistant *Enterobacteriaceae* (CRE) and multi-drug resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [1]. The revival of a once-forgotten antibiotic, colistin, as a cornerstone agent against such pathogens was a necessity [2]. More recently, the discovery of mobile colistin resistance (MCR) genes, signals a new era in antimicrobial resistance, one with potentially significant health burdens, and it is estimated that lives lost annually to resistant and untreatable infections could amount to 10 million by 2050 [3]. The identification of the transferrable MCR genes, and especially in isolated clinically cases, highlight the need for more stringent regulation and monitoring of antimicrobials that are used in both animals and humans.

Colistin (also known as Polymyxin E) is one of two clinically useful options from the polymyxin group of antibiotics, the other being polymyxin B (PMB). Colistin was so named as it was derived from soil-borne *Bacillus polymyxa* subspecies *colistinus* in Japan in 1949 [5]. Two decades later, its clinical use was abandoned mainly due to issues with nephrotoxicity and the preference for aminoglycosides [4-6]. Yet another two decades later, antimicrobial resistance had forced colistin's return into clinical use, and it is currently one of the "reserve group antibiotics" as defined by the World Health Organisation (WHO) Essential Medicines List Access, Watch and Reserve classification [7-8].

The principal concern with the resurrection of colistin (which received marketing approval in the 1950s) is that it was never subjected to modern drug development measures and pharmacokinetic analysis that is undergone by any new antibiotic that enters the market in the 21st century [9-10]. Transmissible colistin resistance genes and the drying reservoirs for treatment of resistant gram-negative infections mean that ample measures should be taken to minimise further resistance to colistin. Adequate dosing is essential to this, and in South Africa, two published dosing guidelines have addressed this issue [9,11].

Parenteral colistin is not registered for use in humans in South Africa; however, Section 21 of Act 101 of 1965 allows the South African Health Products Regulatory Authority (SAHPRA) [previously known as the Medicines Control Council] to provide permission to institutions to import the product under another jurisdiction [9-10].

Many experts prefer to use colistin combination therapy and although specific agents (such as azithromycin, tigecycline, carbapenems, rifampicin and doxycycline) have been used and are effective in-vitro, systematic reviews have shown that the use of intravenous colistin combination therapy was not an associated with lower mortality when compared to intravenous colistin monotherapy [12-13].

Mortality rates and clinical outcomes in the critically-ill population with resistant infections are dependent on a number of factors aside from the choice of antibiotic, and this includes patient factors such as admission to ICU and APACHE scores on admission [14-16].

Clinical response to colistin has been reported to be between 45% and 88% in global studies [14,16]. International mortality outcomes from several observational studies vary widely between 10 – 70% [6,12,15-16]. Data from two South African studies, one in an intensive care unit and the other in private hospitals, showed in-hospital mortality to

be 50% and 30% respectively [14,17]. One of these studies also reported a bacteriological clearance of 50% [14].

Adverse reactions to colistin include nephrotoxicity, neurotoxicity and hypersensitivity reactions; the commonest of which is nephrotoxicity [4]. The incidence of nephrotoxicity in South Africa is not known, and the reversible nephrotoxic potential of colistin is both dose and duration-dependent and varies widely from 20 to 60 per cent [4].

No studies examine the safety and efficacy of colistin in a predominantly HIV infected adult population. One South African study showed HIV prevalence in individuals aged 15 – 49 years to be the highest in the urban informal settlements, with prevalence in males and females to be 22.6 % and 38.1% respectively [18]. This study aimed to describe our experience at a tertiary level facility and evaluated the use of colistin vis-à-vis safety and efficacy.

METHODS

Study design, Study Population and Products Administered

This was a retrospective cohort study of adult patients (≥18 years of age) admitted to the largest African hospital, Chris Hani Baragwanath Academic Hospital (CHBAH), from June 2014 to May 2017, who were treated with intravenous colistin for a minimum of 72 hours for microbiologically documented evidence of a carbapenem-resistant gram-negative infection. Convenience sampling was used to identify patients employing CHBAH pharmacy records of Section 21 applications to the Medicines Control Council (MCC) for colistin use after approval to conduct the study was obtained from the Human Research and Ethics Committee (HREC) of the University of the Witwatersrand (M 170835). These forms were then examined with related CHBAH hospital records and laboratory results regarding demographical, clinical and

microbiological data, including the identification and antibiotic sensitivities of the organisms involved. Colistin use was seen as acceptable in patients who had a positive microbiological culture for a gram-negative organism resistant to carbapenems and sensitive to colistin and who met one or more of the following criteria for sepsis or biochemical signs of infection (temperature $\geq$ 38°C or $\leq$ 36 38°C, an elevated C-reactive protein (CRP), an elevated procalcitonin (PCT), a white cell count (WCC) $\geq$ 12x10^9/L or $\leq$ 4x10^9/L, heart rate $\geq$ 90 beats per minute, presence of purulent sputum or tracheal aspirate, a respiratory rate $\geq$ 20 per minute or necessity for ionotropic support) [16]. South Africa imports a European colistimethate sodium (CMS) product for parenteral use and hence uses the European dosing convention [9-11]. Loading and maintenance colistin doses documented were analysed if appropriately adjusted for weight and renal function, respectively [9,11]. The Defined Daily Dose (DDD) of colistin was calculated to compare amounts of colistin consumed conveniently. DDD = No. of items issued X amount in each vial/WHO DDD measure (WHO DDD measure for parenteral use of colistin is 3 MU) [16,19].

Definitions and Endpoints

Efficacy of colistin was assessed by determining specific outcomes which included 14-day-mortality, clinical and bacteriological clearance.

Clinical clearance was evaluated based on subjective clinical assessments recorded by the attending physician at the end of treatment with colistin as being *favourable, unfavourable* or *indeterminate* [16].

A *favourable outcome* was a composite indicator that included complete or partial resolution of signs and symptoms and improvement of inflammatory biomarkers (WCC, CRP and PCT) after a course of therapy [16,20-21].

Unfavourable outcomes encompassed cases of treatment failure and all-cause 14-day-mortality.

All-cause mortality at day 14 after initiation of treatment was chosen as a measure of an unfavourable outcome as in patients who are critically ill, 7 days is too short to evaluate outcome and 28 days is too long to interpret the cause of mortality [21].

Treatment failure was a composite measure that referred to the persistence of signs and symptoms and persistently elevated inflammatory markers or reoccurrence of signs and symptoms after discontinuation of colistin [16, 20].

An *indeterminate* outcome was defined as the inability to assess clinical clearance [16].

In-hospital mortality, including mortality > 14 days, was also measured as a secondary endpoint to relate our findings to comparator studies that made use of in-hospital mortality as an endpoint [14 – 17].

Microbiological clearance was determined through examining follow-up cultures of patients that received colistin treatment. These were subsequently evaluated as *eradication*, *treatment failure* or *super-infection* [16].

Eradication was defined as bacteriological clearance of the same organism from the same site on a follow-up culture, where available [16, 20].

Cases, where there was persistence of the organism on follow-up microbiological culture, was termed as *treatment failur*e [16, 20].

Situations, where different organisms were isolated from follow up cultures from the same sites, were termed *super-infection* [16].

All cultures and antibiotic susceptibility testing (AST) at CHBAH were done by the National Health Laboratory Service (NHLS) at the hospital. Standard screening methods were used by the laboratory to identify gram-negative organisms isolated from routine cultures. Antibiotic susceptibility testing (AST) for all other antibiotics was

performed by the MicroScan Walkaway (Siemens, New York, USA)© automated system according to manufacturer instructions. A 2016 joint European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) subcommittee recommended broth microdilution (BMD) as the only reliable method to determine colistin susceptibility. Colistin susceptibility testing was done by the Sensititre (Trek, UK)© automated system and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Furthermore, genotypic testing was available from the National Institute of Communicable Diseases (NICD) and helped to provide molecular confirmation.

Safety of colistin use was evaluated by measuring the endpoint of toxicity rates associated with colistin use concerning nephrotoxicity, neurotoxicity and hypersensitivity reactions.

Pre- and post-treatment creatinine clearance (CrCl) based on the Cockcroft-Gault equation was assessed, and a CrCl $\leq$ 60 mL/min was considered abnormal or consistent with renal impairment [11]. In patients with normal renal function at baseline, nephrotoxicity was defined as $\geq$ two-fold increase in serum creatinine from baseline value at any time during colistin treatment [15].

In patients with pre-existing renal failure, nephrotoxicity was defined as $\geq$ than 50% increase in the serum creatinine level from baseline value or a decline in renal function that required renal replacement therapy (RRT) [15].

The attending physician's notes were evaluated for evidence of documented hypersensitivity reaction or neurotoxic events such as neuromuscular blockade, seizures, ataxia, diplopia and paraesthesia [4,16, 22].

STATISTICAL DATA ANALYSIS

Data collected from the datasheet was captured onto Microsoft Excel (Microsoft Corp, USA)® for primary analysis purposes. Demographic and clinical characteristics were expressed as percentages for categorical variables; means or medians were used to express continuous variables and was supplemented by their respective standard deviations (SD) and interquartile ranges (IQR). We used SPSS Statistics software (SPSS Inc., Chicago, IL, USA)® to perform univariate and multivariate data analysis. Univariate analysis was performed to assess factors associated with clinical outcomes and mortality. Significant variables from the univariate analysis were analysed in a multivariate analysis to determine if there was any further significance of associations. For normally distributed continuous variables, we used the ANOVA test to analyse the significance of associations. Chi-square ($\chi 2$) was used to analyse categorical data. P-value < 0.05 denoted significance for all statistical analyses.

RESULTS

The study identified 118 patients treated with colistin and who met the clinical criteria for sepsis outlined above (Figure 1). Patient demographics and comorbidities are depicted in Table 1. The mean age of the patients was 42.4 years (SD $\pm$ 13.73, range 18 – 83 years) and the female: male ratio was 1.1:1.

One hundred and thirteen underlying comorbidities were documented in 78 patients (66.1%), and HIV positivity was reported in 45 patients (38.1% of the cohort and 41.3% of those tested). Of the patients with HIV infection, 25 patients (55%) were taking antiretroviral treatment (ART) and of these, 24 patients (96%) had a documented HIV viral load and virological suppression was reported in 15 of these patients (63%). Other prevalent comorbidities in our cohort included malignancies, cardiovascular disease

and diabetes, which were reported in 21 patients (17.8%), 12 patients (10.2%), 10 patients (8.5%) respectively. Ten patients (8.5%) had been hospitalised within the past three months.

Admission and hospitalisation characteristics are depicted in Table 2. The majority of the admissions were made by the surgical department (n=55, 46.6%), medical department (n=34, 28.8%) and the burns unit (n=23, 19.5%). The median length of hospitalisation was 42.5 days (IQR 27 – 65.8, range 8-166). Twenty-nine patients (24.6%) were admitted to ICU during their stay and treatment with colistin. The mean APACHE II score at the time of admission to ICU was 21 (SD ± 3.34, range 15-28). The mean length of ICU stay was 28 days (SD ± 11.47, range 9-47). Clinical and biochemical features of sepsis are tabulated in Table 3. Common clinical features included heart rate ≥ 90 beats per minute which was documented in 118 patients (100%) and temperature ≥ 38 °C or ≤ 36 °C which was reported in 110 patients (93.2%). Prevalent biochemical features included elevated CRP which was reported in 118 patients (100%), an abnormal WCC that was reported in 101 patients (85.6%) and an elevated PCT that was seen 64 patients (100% of those tested).

A bacterial culture and susceptibility result directed therapy in all 118 patients (Table 4). There was overlap in sources of cultures in 35 patients, of whom 34 patients (97.1%) had two sources culturing the same organism and one patient (2.9%) had three sources culturing the same organism. Pus swabs were noted as a source of culture in 2 patients (1.7%), but in both patients the same organism was cultured on overlapping blood cultures. Bacteraemia was noted in 77 patients (65.3%) who cultured carbapenem-resistant organisms, in whom 50 patients (64.9%) had

Acinetobacter baumannii and 18 patients (23.4%) had *Klebsiella pneumoniae* isolated on blood culture. Furthermore, *A. baumannii* was the commonest organism cultured from all sources and was cultured in 80 patients (67.8%). Forty (26%) of the pre-treatment cultures analysed were polymicrobial and isolated 45 other organisms, of which 15 were gram-negative and 30 were gram-positive organisms.

The specifics of therapy with colistin, including the details of loading and maintenance doses, are presented in Table 5. The median time from obtaining a culture specimen to initiation of therapy was 6 days (IQR 5 – 9 days, range 3 – 29 days) and the median time from a documented positive culture result to initiation of colistin was 2 days (IQR 1 – 4 days, range 1 – 25 days). The mean cumulative colistin Defined Daily Dose (DDD) prescribed was 22.4 (SD ± 14.10), and the mean cumulative DDD missed was 1.39 (SD ± 3.3). All the scheduled doses of colistin were administered in 86 patients (72.9%), and although reasons for missed doses were generally unspecified, stock shortages were documented as a reason for missed doses in 15 patients (12.7%).
A loading dose was administered in 92 patients (78%) of which, 50 patients (54.3%) received a loading dose appropriate to their ideal body weight and 81 patients (88%) received a loading dose of less than 12 million units [9,11]. The maintenance dose was appropriately adjusted to renal function in only 54 patients (54.2%) [9,11]. Combination therapy with colistin was used in 84 patients (71.2%) and of these, a carbapenem (meropenem or imipenem) was used in 72 patients (85.7%), an aminoglycoside (amikacin) was used in 7 patients (8.3%), piperacillin/tazobactam was used in 2 patients (2.4%), and a cephalosporin (ceftriaxone or cefepime) was used in 3 patients (3.6%). In 39 of the 118 patients (33.1%), antimicrobials were prescribed in addition to the treatment of gram-negative infections, and of these, 25 patients (64.1%)

received vancomycin for therapy of gram-positive infections, 7 patients (17.9%) were on anti-tuberculosis treatment, 6 patients (15.4%) were on treatment with anti-fungal agents and one patient (2.7%) was receiving anti-viral treatment with acyclovir.

We were able to make assessments of clinical outcome (Table 6) in all patients, and hence there were no indeterminate results. There was a documented resolution of clinical signs in 70 patients (59.3%) that was consistent with a favourable outcome. Treatment failure was documented for 11 patients (9.3%), and in these patients, there was a failed resolution of clinical symptoms. Fourteen-day-mortality was seen in 37 patients (31.4%) of whom 23 patients (62.2%) died within 7 days. Unfavourable outcome (a composite of treatment failure and 14-day- mortality) was documented in 48 patients (40.7%). In-hospital mortality was documented in 43 patients (36.4%) of which, 6 patients died after 14 days. The median days to death was 7 days (IQR 5 – 12 days, range 3-37). Univariate analysis done to assess significant factors associated with outcome (Table 7) found that independent factors associated with a favourable outcome included younger age (p-value = 0.020), male gender (p-value = 0.026), increased length of hospitalisation (p-value = 0.000), the use of combination therapy (p-value = 0.008), the use of a loading dose (p-value = 0.014), the total DDD administered (p-value = 0.000), if a follow-up culture was done (0.000) and if the patient did not develop nephrotoxicity (0.001). A multivariate analysis of these significant variables using ordinal logistic regression was done (Table 8) and it showed that male gender [OR 3.269, CI 1.465 – 7.295 (p-value 0.004)], the presence of a follow-up culture [OR 12.870, CI 4.335 – 38.206 (p-value 0.000)] and the absence of nephrotoxicity [OR 0.248, CI 0.123 – 0.496 (p-value 0.000)] to be still statistically significant.

We were able to assess microbiological clearance in 95 patients (80.5%) as follow-up culture was done during or post treatment with colistin (Table 6). Microbiological eradication of the resistant organism was achieved in 85 patients (89.5%), of which super-infection was documented in 14 patients (16.5%), and in-hospital mortality was documented in 15 patients (17.6%). Microbiological treatment failure was documented in 10 patients (10.5%), and of these, 7 patients (70%) had documented in-hospital mortality. A review of these 10 patients also showed that, although all doses of treatment were administered, only two of these patients (20%) received appropriate dosing of treatment – 6 patients (60%) did not receive a loading dose and 5 patients did not receive a maintenance dose in compliance with the guidelines [9,11]. Furthermore, of the 10 patients who had microbiological treatment failure, 6 patients (60%) also had a documented polymicrobial culture with a gram-positive organism.

Nephrotoxicity was documented in 37 patients (31.4%) receiving treatment with colistin (Table 9). Eighty-six patients (72.9%) had a normal pre-treatment CrCl and 26 of these patients (30.2%) had a two-fold or greater increase in creatinine during/after treatment and 3 of these patients (11.5%) required haemodialysis.

Thirty-two patients (27.1%) in our cohort had renal impairment prior to treatment, and of these, 7 patients (21.9%) had established CKD with kidney failure as per the Kidney Disease Improving Global Outcomes (KIDGO) classification, and the remaining 25 patients (78.1%) had a normal renal function on admission to hospital but had developed renal impairment prior to treatment with colistin. Of the 32 patients who had renal impairment prior to treatment, 11 patients (34.3%) developed nephrotoxicity, and 10 of these patients (90.9%) received haemodialysis. The need for dialysis was greater in those with an abnormal pre-treatment CrCl compared to those with a normal

pre-treatment CrCl and this difference was statistically significant [OR 12.58, CI 3.19 – 49.65 (p-value 0.0003)].

The median pre-treatment CrCl was 96 mL/min (IQR 54.8 – 151.5 mL/min, range 15 – 355 mL/min) and the median post-treatment CrCl was 74 mL/min (IQR 36.3 – 123 mL/min, range 8 – 387 mL/min). The median pre-treatment creatinine was 77μmol/L (IQR 52 – 144.5 μmol/L and range 30 – 757 μmol/L) and the median post-treatment creatinine was 97.5 μmol/L (IQR 57.5 – 189.5 μmol/L and range 26 – 1558 μmol/L). Additional nephrotoxins were noted to be used in addition to colistin in 44 patients (37.2%) during treatment and the frequency of these agents are illustrated in Figure 2. Independent variables associated with nephrotoxicity identified in a univariate analysis included *Staphylococcus aureus* cultured on polymicrobial culture (p-value = 0.041), unfavourable clinical outcome (p-value = 0.001), nephrotoxins used (p-value = 0.013), and in-hospital mortality (p-value = 0.013). Multivariate analysis of these variables using binary logistic regression (Table 10) showed *Staphylococcus aureus* cultured on polymicrobial culture [OR 21.841, CI 1.589 – 298.419 (p-value 0.021)], 14-day-mortality [OR 67.995, CI 3.317 – 1392.070 (p-value 0.006)] and treatment failure [OR 25.136, CI 3.568 – 177.095 (p-value 0.001)] to be statistically significant.

Although nephrotoxicity was inferred from changes in serum creatinine and the need for dialysis, the other features of colistin toxicity required a more challenging retrospective assessment of patient records for documentation of such toxicities by the attending physician during the time of the event. A type 2 error may exist as there were no documented reports of neurotoxicity and hypersensitivity reactions with colistin in our study.

Univariate analysis showed a significant difference in the mean age between those who were HIV seropositive (38.7 years ± SD 9.95 years) compared to those who were HIV seronegative (43.9 years ± SD 15.01 years) [p-value = 0.030]. The shorter length of hospital stays in those who were HIV seropositive (48.7 days ± SD 28.7 days) compared to those who were HIV seronegative (54.9 days ± SD 33 days) was also shown to be significant [p-value = 0.010]. However, the significance of these associations was not replicated in a multivariate analysis. Univariate analysis failed to show associations between HIV seropositivity and clinical and microbiological outcomes and in-hospital mortality.

DISCUSSION

There is limited data regarding the effectiveness of colistin concerning clinical and microbiological clearance and toxicity in the South African setting [14,22]. Management of antimicrobial resistance associated with MDR-GNB has been a significant public health concern, and this has been mitigated through the release of two South African guidelines regarding the use of colistin [9,11]. Given that colistin's use is not ubiquitous and the recent advances in our understanding of its pharmacodynamics and kinetics, such guidelines are even more pertinent in any setting.

This audit revealed that clinical clearance was documented in the majority of patients (58.4%) and was comparable with clinical clearance reported in the literature of between 45% and 88% [14,16]. Of the patients who achieved clinical clearance, a favourable microbiological clearance was also documented in 64 patients (91.4%). A limitation to the assessment of microbiological clearance was that, although a

laboratory directed culture was used to guide therapy in all patients (n=118, 100%), follow-up bacterial culture was performed in only 95 patients (80.5%).

Unfavourable outcome measures included 14-day-mortality and treatment failure, and this was seen in 48 patients (40.7%). Fourteen-day-mortality was documented in 37 patients (31.4%) and 6 patients (5.1%) died after day fourteen and 2 of these patients (33.3%), died after 28 days. In-hospital mortality, evaluated a secondary outcome measure, was seen in 43 patients (36.4%) and was comparable to South African in-hospital mortality rates reported in the literature of between 30 and 50% [14,17].

Many experts recommend the use of combination therapy with colistin despite lack of conclusive evidence of better outcomes, and there is currently an ongoing debate as to whether combination therapy is superior to colistin monotherapy. In this study, combination therapy was used in 84 patients (71.2%) and carbapenems (imipenem or meropenem) were used in the majority of cases (n=72, 85,7%). This is in keeping with a global survey of colistin use that showed that clinicians frequently used carbapenems in combination with colistin and selected the second antibiotic depending on the type of infection and susceptibility profiles of the cultured bacteria [23]. Univariate analysis signified an association between combination therapy and clinical outcome; however, there was no significance of association in the multivariate analysis. The in-vitro success of specific agents used in combination with colistin has not been conclusively replicated in clinical practice and although a number of observational studies that have shown higher rates of survival for combination treatment in patients with MDR-GNB infections, it is contrary to what has been shown in multiple RCTs according to recent systematic reviews [12-13, 24].

Factors that were positively associated with a favourable clinical outcome included male gender [OR 3.269, CI 1.465 – 7.295 (p-value 0.004)] and the presence of a

follow-up culture [OR 12.870, CI 4.335 – 38.206 (p-value 0.000)]. Males were three times as likely to have a favourable outcome, when compared to females and patients who had a follow-up culture done were 13 times as likely to have a favourable outcome when compared to those who had no follow-up culture done. It is important to note that a type 1 error may exist concerning follow-up for non-bacteraemic cultures as patients with favourable clinical outcome may have had initial culture indicating colonisation rather than infection. Despite this confounder, follow-up cultures are pertinent in the management of such patients and especially so in those failing to respond to treatment.

HIV seropositivity was a significant comorbidity in our patients and was documented in 45 patients (38.1%), of which only 25 patients (55.6%) were taking antiretroviral treatment (ART). Despite the low median CD4 count of 136 cells/μL (IQR 36 – 286 cells/μL, range 1 – 1454 cells/μL) and hence the increased susceptibility to opportunistic infections, logistic regression analysis failed to find an association of HIV seropositivity with clinical outcome, microbiological outcome, in-hospital mortality or nephrotoxicity. However, a type 2 error may exist from the small sample size of our HIV seropositive population and further longitudinal studies may be needed in this regard.

This study population is unique due to higher HIV seropositivity as well as a much younger cohort (mean age 42.4, SD ± 13.73, range 18 – 83) than seen in other international studies [6,15-16]. In our setting, with our high HIV burden, other considerations may play a compounding role in adverse outcomes such as the many drug interactions that exist in patients on numerous medications, the metabolism of colistin in HIV positive individuals and the increased susceptibility to opportunistic and

nosocomial infections [22]. These factors were not explored in our study and underscore the importance of further studies, especially in our patient demographic.

Nephrotoxicity due to colistin use was assessed in this study and found in 37 patients (31.4%). Although similar rates of nephrotoxicity were seen in those with a normal pre-treatment CrCl (30.2%) and those with an abnormal pre-treatment CrCl (34.4%), the need for dialysis was greater in those with an abnormal pre-treatment CrCl [OR 12.58, CI 3.19 – 49.65 (p-value 0.0003)]. It is of note that a type 1 error may exist as pre-existing renal dysfunction is itself a risk factor for nephrotoxicity. Data regarding nephrotoxicity in patients treated with colistin suggest a considerable variation in the nephrotoxic potential of this antibiotic. One recent review reports the nephrotoxic potential of colistin to be between 20% and 60% [4]. Reportedly, the toxicity potential had also significantly declined over time from when colistin was initially adopted, and factors to account for this include the use of CMS instead of colistin sulphate, improved purification methods, guidelines for dose adjustments in renal failure and increased awareness for nephrotoxicity with other agents [12]. Concomitant nephrotoxins were noted to be used in 44 patients (37.2%), of which, vancomycin was used in 25 patients (56.8%). Binary logistic regression showed that those with *Staphylococcus aureus* cultured on polymicrobial culture were significantly associated with nephrotoxicity (Table 10). A review of the 8 patients who cultured *Staphylococcus aureus* (of which, only 4 patients had cultured methicillin-resistant *Staphylococcus aureus* (MRSA)) showed that 7 patients (87.5%) were treated concomitantly with vancomycin and 5 patients (62.5%) developed nephrotoxicity. This highlights the concomitant nephrotoxic potential of other antimicrobials such as vancomycin as well as the importance of prescribing rationally. Furthermore, binary logistic regression showed

that unfavourable clinical outcome was also significantly associated with nephrotoxicity (Table 10). Aside from the many confounding factors in critically ill patients (such as organ dysfunction in sepsis, the effect of nephrotoxic agents and severity of patient illness), discrepancies in nephrotoxic potential may also be due to variable definitions of nephrotoxicity adopted by researchers over the years as well as lack of a standardised dosing scheme [4, 25]. Thus, the importance of prospective studies using standardised criteria such as the RIFLE criteria (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) or the Acute Kidney Injury Network (AKIN) classification cannot be more unequivocally stated. We found that in 26 patients (22%) with normal pre-treatment renal function, a two-fold or greater increase in creatinine was noted during treatment with colistin and these patients could also be classified as having Stage 2 or higher post-treatment AKIN scores which correlate with Injury or higher post-treatment RIFLE scores [26]. However, due to the limitations of being a retrospective audit, a more comprehensive assessment of acute kidney injury could not be made.

In trying to optimise the safety and efficacy of colistin, we should also question the status quo and ask if colistin is genuinely the best option in treating organisms susceptible to polymyxins. PMB may be a better alternative as it is associated with lower rates of nephrotoxicity and is eliminated via non-renal mechanisms [27]. Despite having identical chemical structures and having similar mechanisms of action, resistance patterns and spectrum of activity, PMB and colistin differ significantly in their pharmacodynamic and pharmacokinetic properties [4]. The advantage of PMB is its ability to rapidly provide predictable serum concentrations without the need for conversion into an active form, renal adjustments and at a lower cost [27, 28]. Despite

this, efficacy of colistin is equal to that PMB and studies have shown that there is no difference in mortality [29].

Aside from safety and efficacy parameters, this audit identified numerous key factors in the use of colistin at CHBAH which included prescribing patterns, adherence to treatment guidelines, dosing parameters, microbiological confirmation and time to treatment. Regular reviews, such as this study, provide opportunities to improve antimicrobial stewardship (AMS) at CHBAH. This study found that the surgical wards and the burns unit accounted for the majority of the admissions (n= 78, 66%), which suggests opportunities for improved infection prevention control and antimicrobial stewardship in these areas. This is consistent with a recent study in South African tertiary hospitals exploring the clinical characteristics of carbapenem resistant Enterobacteriaceae (CRE) bacteraemia which was common amongst those with surgical admissions (including those with skin and soft tissue infections) [30]. A multidisciplinary approach to AMS involving partners from pharmacy, clinical microbiology, infectious diseases, nursing staff and treating clinicians has proven to reduce antimicrobial use and costs without compromising patient outcome in our setting [31].

This study noted that a loading dose was administered in 92 patients (78%) of which, 50 patients (54.3%) received a loading dose appropriate to their ideal body weight and 81 patients (88%) received a loading dose of less than 12 million units [9,11]. This indicated a failure to comply with available guidelines since both guidelines recommend using high loading doses (9 – 12 MU) in all patients, regardless of renal function, for rapid attainment of the recommended minimum inhibitory concentration (MIC) breakpoint [9,11].

The maintenance dose was appropriately adjusted to renal function in only 54 patients (54.2%) [9,11]. The significantly large variability in maintenance doses may result from confusion surrounding the available guidelines or a failure to comply with them. Both guidelines use different strategies to tier GFR to maintenance dose recommendations [9,11]. The South African Society of Clinical Pharmacy (SASOCP) guideline employs creatinine clearance (CrCl) using the Cockcroft-Gault formula to evaluate the glomerular filtration rate as opposed to estimated glomerular filtration rate (e-GFR) using either Modification of Diet in Renal Disease (MDRD) or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas. Although the MDRD or the newer CKD-EPI formula produces more accurate estimates of GFR in comparison to CrCl estimates using the much older Cockcroft-Gault equation, using CrCl remains a viable strategy for dose adjustments in renal failure as most drugs are validated using this method.

Failure of compliance to published guidelines may suggest a lack of awareness of such guidelines and an opportunity for improved education in this regard [9,11]. The addition of a colistin dosing guideline to the existing CHBAH AMS committee's empiric antibiotics guidelines on the mobile and web-based medicines and treatment platform, EM Guidance, could be considered as a reference tool for clinicians [32]. Furthermore, prescriber education through AMS ward rounds and the re-enforcement of such guidelines is pertinent in ensuring optimal dosing.

Delays in initiation of treatment with colistin may result in adverse consequences, especially in critically ill patients, such as those with septic shock, who have a greater risk of mortality than sepsis alone [33]. Such delays may be due to drug availability, logistical or administrative challenges, delays in microbiological confirmation, poor

communication or notification of such results and failure of treating clinicians to follow-up cultures timeously.

This study showed that all scheduled doses of colistin were administered in 86 patients (72.9%), and although reasons for missed doses were generally unspecified, stock shortages were documented as a reason for missed doses in 15 patients (12.7%) and this may be as a result of administrative or logistical issues. Many studies both locally and internationally reported barriers to access colistin (including the administrative challenges such as the tedious application for the use of colistin) as obstructive in the treatment of critically ill patients [10, 34]. In South Africa, use is governed by the SAHPRA through Section 21 of Act 101 of 1965 [9 – 10]. Additionally, since a copy of the microbiology culture report is a requirement for the application process in South Africa, this prevents the use of the antibiotic as empirical therapy. Such legislative processes are aimed at promoting the judicious use and stewardship of colistin. The dichotomy between using empiric antibiotics of last resort in the treatment of critically ill patients and legislative barriers to restrict the access of such therapies as part of antimicrobial stewardship will always create friction, and this is healthy. However, a solution to reducing delays may be to fast-track approval and acquisition of the drug in patients when recommended by preliminary microbiological reports suggesting carbapenem resistance prior to confirmatory phenotypic and genotypic tests.

The use of empirical antimicrobial therapy (EMAT) with colistin is controversial and should be appropriate to the setting in which it is used further substantiating a multidisciplinary AMS approach. A recent 2018 study showed that the empirical use of colistin (before pathogen recognition) was associated weakly with mortality and that there was no association with survival in patients with carbapenem resistant gram-negative bacteria [35].

This retrospective audit showed that the median time from performing a culture to treatment was found to be 6 days (IQR 5 – 9, range 3 – 29), of which the median delay after a positive culture was 2 days. Although the median time from performing a culture to treatment was not significant for an unfavourable clinical outcome, all patients in this audit had delays in treatment and we were unable to make an appropriate comparison. Such delays could be avoided through use of standardised protocols for notifying and following-up of microbiological results. Furthermore, communication between clinical and microbiological staff is essential in the management of critically ill patients as treating clinicians should raise concerns of possible suboptimal infection management and appropriateness of therapy and microbiologists should inform clinicians of any pertinent preliminary microbiology results or delays in obtaining culture results. The identification and resolution of such issue's emphasises the need for a multidisciplined approach to AMS.

As part of the many limitations of doing an observational retrospective audit, bias may interfere with our evaluation of the effectiveness of colistin concerning clinical and microbiological clearance and toxicity. This study had a small sample size and was conducted in a single public facility. Application forms to SAPHRA were used in the identification of patients in this study, however of the 178 patients identified, only 128 patients' hospital records were found, implying that almost a third of identified patients' records were missing. Both missing data and patient records lower a study's statistical power and contribute to bias.

CONCLUSION

We have managed to show that colistin is clinically and microbiologically effective in the treatment of MDR-GNB in our setting and that the nephrotoxicity potential of colistin was similar to globally experienced rates. This audit has identified numerous opportunities to optimise the AMS of colistin at CHBAH and substantiates the need for further audits of this nature to ensure that last resort antimicrobials, such as colistin, are being used effectively and sparingly.

The authors have no conflicts of interest to declare.

Table 1. Patient Demographics and Comorbidities	
Demographic Criteria	
Age (in years) (mean ± SD, range)	42.4 ± 13.73, 18 – 83
Female gender [*n* (%)]	62 (52.5)
Male gender [*n* (%)]	56 (47.5)
Underlying Co-morbidities (*n* =78)	
HIV positivity total [*n* (%)]	45 (38.1)
HIV positive on ART [*n* (%)]	25 (21.2)
HIV positive not on ART [*n* (%)]	20 (16.9)
HIV status unknown [*n* (%)]	9 (7.6)
CD_4 cell count (cells/μL) (median, IQR, range)$^{\lambda}$	136, 36 – 286, 1 – 1454
HIV viral load (copies/mL) (median, IQR, range)$^{\theta}$	26, 45 – 146332, 0 – 10000000
Solid malignancies [*n* (%)]	13 (11.1)
Cardiovascular disease (including hypertension) [*n* (%)]	12 (10.2)
Diabetes [*n* (%)]	10 (8.5)
Haematological malignancies [*n* (%)]	8 (6.8)
Neurological disease [*n* (%)]	7 (5.9)
CKD [*n* (%)]	7 (5.9)
Chronic respiratory illness [*n* (%)]	6 (5.1)
Immunosuppressive treatment [*n* (%)]	5 (4.2)
Recent admission$^{\varepsilon}$ [*n* (%)]	10 (8.5)

SD – Standard deviation; HIV – Human immunodeficiency virus; ART – Anti-retroviral therapy; CD_4 – Cluster of differentiation 4; CKD – Chronic kidney disease; RRT – Renal replacement therapy

λ CD_4 cell count during admission, results only available for 41 of 45 HIV positive patients

θ HIV viral load during admission, results only available for 40 of 45 HIV positive patients

ε Recent hospitalisation within the past three months

Table 2. Patient Hospitalisation and Admission Characteristics	
Hospitalisation	
Length of hospitalisation (in days) (median, IQR, range)	42.5, 27 – 65.8, 8 – 166
ICU admission [*n* (%)]	29 (24.6)
Length of ICU stay (in days) (mean ± SD, range)	28 ± 11.47, 9 – 47
APACHE II score on ICU admission (mean ± SD, range)	21 ± 3.34, 15 – 28
Admitting Departments [n (%)]	**118 (100)**
Surgical wards [*n* (%)]	55 (46.6)
Medical wards [*n* (%)]	34 (28.8)
Burns ICU [*n* (%)]	23 (19.5)
Obstetrics and Gynaecology wards [*n* (%)]	6 (5.1)

SD – Standard deviation; ICU – Intensive Care Unit; APACHE II – Acute Physiology and Chronic Health Evaluation II

Table 3. Frequency of Pre-treatment Clinical Features of Infection (n=118)	
Temperature ≥ 38 °C or ≤ 36 °C [*n* (%)]	110 (93.2)
WCC < 4000/mm³ or > 12000/mm³ [*n* (%)]	101 (85.6)
WCC (x 10^9/L) (median, IQR, range)	14.18, 10.59 – 17.59, 0.15 – 70.53
Heart rate ≥ 90 beats per minute [*n* (%)]	118 (100)
Respiratory rate ≥ 20 breaths per minute [*n* (%)]	27 (22.9)
Necessary inotropic support [*n* (%)]	26 (22)
Purulent sputum or tracheal aspirate [*n* (%)]	15 (12.7)
Elevated CRP [*n* (%)]	118 (100)
CRP (mg/dL) (median, IQR, range)	195, 123 – 289, 18 – 487
Elevated PCT [*n* (%)]	64 (54.2)
PCT (ng/mL) (median, IQR, Range)$^{\alpha}$	5.35, 2.5 – 26.5, 0.25 – 239.8

°C – degrees Celsius; WCC – White cell count; CRP – C-reactive protein; PCT – Procalcitonin

α PCT value was only documented in 64 patients and was missing for 54 patients

Table 4. Cultures and Spectrum of Organisms	
Sources of Cultures Analysed[α] [n (%)]	**154 (100)**
Bloodstream [*n* (%)]	77 (65.3)
Arterial catheter tip [*n* (%)]	21 (17.8)
Sputum/Tracheal aspirate [*n* (%)]	13 (11)
Urine [*n* (%)]	12 (10.2)
Tissue [*n* (%)]	11 (9.3)
Fluid aspirate [*n* (%)]	11 (9.3)
Pus [*n* (%)]	3 (2.5)
Wound pus swab [*n* (%)]	2 (1.7)
CSF [*n* (%)]	2 (1.7)
Other/Unspecified[*] [*n* (%)]	2 (1.7)
Polymicrobial Cultures [*n* (%)]	40 (26)
Spectrum of Carbapenem Resistant Organisms Cultured [n (%)]	**118 (100)**
***Acinetobacter baumannii* [*n* (%)]**	80 (67.8)
***Klebsiella pneumoniae* [*n* (%)]**	23 (19.5)
***Escherichia coli* [*n* (%)]**	8 (6.8)
***Pseudomonas aeruginosa* [*n* (%)]**	4 (3.4)
***Klebsiella oxytoca* [*n* (%)]**	3 (2.5)
Organisms Isolated from Polymicrobial Cultures [n (%)]	**45 (100)**
Gram-positive organisms isolated [*n* (%)]	30 (66.7)
Gram-negative organisms isolated [*n* (%)]	15 (33.3)
CSF – Cerebrospinal fluid	
α	There was overlap in the sources of cultures in 35 patients of whom 34 patients had 2 sources culturing the same organism and 1 patient had 3 sources culturing the same organism.
*	Included a synovial fluid specimen in a patient with septic arthritis and cerebrospinal fluid specimen in a patient with ventriculitis.

Table 5. Specifics of Therapy with Colistin	
Duration of Non-treatment[ν]	
Time to positive culture (in days) (median, IQR, range)	4, 3 – 4, 1 – 11
Time from positive culture result to treatment (in days) (median, IQR, range)	2, 1 – 4, 1 – 25
Time from obtaining culture specimen to treatment (in days) (median, IQR, range)	6, 5 – 9, 3 – 29
Cumulative DDD Prescribed and Administered[θ]	
Cumulative DDD prescribed (mean ± SD)	22.4 ± 14.1
Cumulative DDD administered (mean ± SD)	21 ± 11.9
Cumulative DDD missed (mean ± SD)	1.39 ± 3.3
Loading Dose Prescribed [n (%)]	**92 (100)**
2 MU [*n* (%)]	1 (0.8)
3 MU [*n* (%)]	11 (9.3)
6 MU [*n* (%)]	3 (2.5)
9 MU [*n* (%)]	66 (55.9)
12 MU [*n* (%)]	11 (9.3)
Loading dose appropriate to weight [*n* (%)][μ]	50 (42.4)
Maintenance Dose Prescribed [*N* (%)]	**118 (100)**
1 MU BD [*n* (%)]	8 (6.8)
1 MU TDS [*n* (%)]	15 (12.7)
2 MU BD [*n* (%)]	11 (9.3)
2 MU TDS [*n* (%)]	2 (1.7)
3 MU OD [*n* (%)]	1 (0.8)
3 MU OD [*n* (%)]	10 (8.5)
3 MU TDS [*n* (%)]	19 (16.1)
4.5 MU BD [*n* (%)]	52 (44.1)
Maintenance dose appropriate to renal function [*n* (%)][μ]	64 (54.2)
Combination therapy administered [*N* (%)]	**84 (100)**
Carbapenems (imipenem and meropenem) [*n* (%)]	72 (85.7)
Aminoglycosides (amikacin) [*n* (%)]	7 (8.3)
Piperacillin/tazobactam [*n* (%)]	2 (2.4)
Cephalosporins (ceftriaxone and cefepime) [*n* (%)]	3 (3.6)
Other Antimicrobials Used in Conjunction [*N* (%)]	**39 (100)**
Therapy directed at gram-positive infections [*n* (%)]	25 (64.1)
Anti-tuberculosis treatment [*n* (%)]	7 (17.9)
Anti-fungal agents [*n* (%)]	6 (15.2)
Anti-viral agents [*n* (%)]	1 (2.7)

SD – Standard deviation; DDD – Defined daily dose; MU – Million Units; BD – Dosed bi-daily; OD – Dosed once-daily; TDS – Dosed trice-daily; WHO – World Health Organisation
ν Median times in days from obtaining culture specimen to culture positivity and from culture positivity to initiation of therapy with colistin.
θ Defined Daily Dose = No. of items issued X amount in each vial/ WHO DDD measure (WHO DDD measure for parenteral use of colistin is 3 MU).
μ In compliance with either the South African Society for Clinical Pharmacy guideline or the Visser Kift *et al* guideline.[9,11]

Table 6. Clinical Outcome, Mortality and Microbiological Clearance	
Clinical Outcome [n (%)]	**118 (100)**
Favourable outcome [*n* (%)]	**70 (59.3)**
Unfavourable outcome [*n* (%)]	**48 (40.7)**
Treatment failure [*n* (%)]	11 (9.3)
14-day-mortality [*n* (%)]	37 (31.4)
Indeterminate outcome [n (%)]	0 (0)
Mortality [n (%)]	**43 (100)**
7-day-mortality [*n* (%)]	23 (53.4)
14-day-mortality [*n* (%)]	37 (86)
28-day-mortality [*n* (%)]	41 (95.3)
Death after day 28 [*n* (%)]	2 (4.65)
Time to death$^{\Omega}$ (in days) (median, IQR, range)	7, 5 – 12, 3 – 37
Microbiological Clearance [n (%)]	**95 (100)**
Eradication *[n (%)]*	71 (74.7)
Treatment failure *[n (%)]*	10 (10.5)
Superinfection *[n (%)]*	14 (14.7)
Microbiological and Clinical Clearance ^{X} [n (%)]	**64 (54.2)**
IQR – Inter-quartile range	
Ω Mean days to death from initiation of therapy with colistin.	
X Documented clinical and microbiological clearance in *N*=118 patients.	

Table 7. Univariate Analysis of Variables Associated with Favourable and Unfavourable Response				
	Favourable Response (*N* = 70)	Unfavourable Response (*N* = 48)		
		Treatment Failure (*N* = 11)	14-day-mortality (*N* = 37)	p-value
DEMOGRAPHIC FACTORS				
Age (in years) (mean ± SD)	39.5 ± 12.9	48.2 ± 11.8	46.1 ± 14.7	**0.020**
Male Gender$^{\bullet}$ [*n* (%)]	40 (57.1)	5 (45.5)	11 (29.7)	**0.026**
Weight (in kg) (mean ± SD)	72.5 ± 18	74.3 ± 10	72.4 ± 20	0.949
ICU admission [*n* (%)]	19 (27.1)	3 (27.3)	7 (18.9)	0.628
APACHE II Score$^{\Delta}$ (mean ± SD)	19.9 ± 3	22.7 ± 5	21.9 ± 3	0.232
Length of hospitalisation (in days) (mean ± SD)	59 ± 34	49.8 ± 14	33.1 ± 23	**0.000**
CO-MORBID CONDITIONS				
CKD TOTAL [*n* (%)]	2 (2.6)	1 (9.1)	4 (10.8)	0.120
CKD not on chronic dialysis [*n* (%)]	1 (1.4)	1 (9.1)	4 (10.8)	0.090
CKD on chronic dialysis [*n* (%)]	1 (1.4)	0	0	0.708
HIV TOTAL [*n* (%)]	29 (41.4)	6 (54.5)	10 (27)	0.249
HIV not on treatment [*n* (%)]	12 (17.1)	2 (18.2)	6 (16.2)	0.986
HIV on treatment [*n* (%)]	17 (24.3)	4 (36.4)	4 (10.8)	0.166
Haematological malignancy [*n* (%)]	5 (7.1)	0	3 (8.1)	0.632
Non-haematological malignancy [*n* (%)]	5 (7.1)	3 (27.3)	5 (13.5)	0.188
Respiratory disease [*n* (%)]	4 (5.7)	1 (9.1)	1 (2.7)	0.651
Cardiac disease [*n* (%)]	4 (5.7)	3 (27.3)	5 (13.5)	0.064
Neurological disease [*n* (%)]	5 (7.1)	0	2 (5.4)	0.639
Diabetic [*n* (%)]	5 (7.1)	1 (9.1)	4 (10.8)	0.808
Use of immunosuppressive agents [*n* (%)]	3 (4.3)	0	2 (5.4)	0.737

Recent admission in the past three months [*n* (%)]	4 (5.7)	2 (18.2)	4 (10.8)	0.319
SITES OF CULTURES				
Bloodstream [*n* (%)]	48 (68.6)	7 (63.6)	22 (59.5)	0.637
Arterial Catheter Tip [*n* (%)]	12 (17.1)	1 (9.1)	8 (21.6)	0.619
Sputum and Tracheal Aspirate [*n* (%)]	9 (12.9)	1 (9.1)	3 (8.1)	0.740
Urine [*n* (%)]	7 (10)	1 (9.1)	4 (10.8)	0.948
Tissue [*n* (%)]	5 (7.1)	1 (9.1)	5 (13.5)	0.559
Fluid Aspirate [*n* (%)]	6 (8.6)	1 (9.1)	4 (10.8)	0.930
Pus [*n* (%)]	2 (2.9)	0	1 (2.7)	0.853
Wound Pus Swab [*n* (%)]	2 (2.9)	0	0	0.498
CSF [*n* (%)]	1 (1.4)	1 (9.1)	0	0.118
Other/ Unspecified [*n* (%)]	1 (1.4)	0	1 (2.7)	0.801
SPECTRUM OF ORGANISMS				
***Acinetobacter baumannii* [n (%)]**	45 (64.3)	9 (81.8)	26 (70.3)	0.475
***Klebsiella pneumoniae* [n (%)]**	16 (22.9)	2 (18.2)	5 (13.5)	0.895
***Escherichia coli* [n (%)]**	4 (5.7)	0	0	0.721
***Pseudomonas aeruginosa* [n (%)]**	2 (2.9)	0	2 (1.7)	0.867
***Klebsiella oxytoca* [n (%)]**	3 (2.5)	0	0	0.348
***Enterobacter cloacae* [n (%)]**	0	0	0	0.118
THERAPY RELATED FACTORS				
Polymicrobial culture♣ [*n* (%)]	24 (34.2)	5 (45.5)	17 (45.9)	0.450
Combination therapy♦ [*n* (%)]	57 (81.4)	5 (45.5)	22 (59.5)	**0.008**
Loading dose♥ [*n* (%)]	59 (84.3)	5 (45.5)	28 (75.7)	**0.014**
Appropriate adjustment of loading dose [*n* (%)]	35 (50)	2 (18.2)	13 (35.1)	0.078
Appropriate adjustment of maintenance dose [*n* (%)]	40 (57.1)	4 (36.4)	20 (54.1)	0.068
Total duration of non-treatment (in days) (mean ± SD)	7.3 ± 2.9	6.8 ± 1.4	8.6 ± 5.5	0.128
Duration of treatment (in days) (mean ± SD)	9.4 ± 4.3	11.5 ± 5.5	10.5 ± 3.6	0.226
Total DDD administeredΦ (mean ± SD)	23.3 ± 10.6	27 ± 20.5	14 ± 7.8	**0.000**
Total DDD missed (mean ± SD)	1.9 ± 4.5	3.3 ± 5	0.9 ± 0.1	0.215
Follow-up culture [*n* (%)]	67 (95.7)	11 (100)	17 (45.9)	**0.000**
Pre-treatment CrCl (in mL/min) (mean ± SD)	122.8 ± 82.5	92.7 ± 55	92.4 ± 50.6	0.084
Nephrotoxicity [*n* (%)]	13 (18.5)	7 (63.6)	17 (45.9)	**0.001**
Nephrotoxins used [*n* (%)]	22 (31.4)	4 (36.4)	17 (45.9)	0.332

SD - Standard deviation; ICU – Intensive care unit; APACHE II – Acute Physiology and Chronic Health Evaluation II; CKD – Chronic Kidney Disease; HIV – Human Immunodeficiency Virus; CSF – Cerebrospinal fluid; DDD – Defined daily dose; CrCl – Creatinine clearance; WHO – World Health Organisation

∗ The values for female gender are inferred

Δ APACHE II Score on day of ICU admission (for ICU patients only)

♣ Polymicrobial culture documented

♦ The use of combination therapy

♥ The use of a loading dose

Φ Defined Daily Dose = No. of items issued x amount in each vial/WHO DDD measure (WHO DDD measure for parenteral use of colistin is 3 MU)

Table 8. Multivariate Analysis of Variables Associated with Favourable Outcome		
Variables	**OR (95% CI)**	**p-value**
Age	0.985 (0.962 – 1.009)	0.219
Male gender	3.269 (1.465 – 7.295)	0.004
Length of hospitalisation	1.010 (0.996 – 1.023)	0.155
Combination therapy	1.269 (0.675 – 2.386)	0.459
Loading dose	1.083 (0.515 – 2.287)	0.834
Total DDD administered	1.014 (0.989 – 1.040)	0.263
Follow-up culture	12.870 (4.335 – 38.206)	0.000
Absence of nephrotoxicity	0.248 (0.123 – 0.496)	0.000
OR – Odds ratio; CI – Confidence interval; DDD – Defined daily dose		

Table 9. Nephrotoxicity Associated with Colistin	
Abnormal Pre-treatment CrCl$^{\forall}$ [n (%)]	32 (100)
Established CKD [*n* (%)]	7 (21.9)
Established CKD not on RRT pre-treatment$*$ [*n* (%)]	6 (18.8)
Established CKD on RRT pre-treatment$^{\ni}$ [*n* (%)]	1 (3.1)
Normal renal function at admission but abnormal pre-treatment CrCl$^{\forall}$ [*n* (%)]	25 (78.1)
Nephrotoxicity$^{\wedge}$ [*n* (%)]	11 (34.3)
Required dialysis	10 (31.3)
Normal Pre-treatment CrCl [n (%)]	86 (100)
Nephrotoxicity$^{\#}$ [*n* (%)]	26 (30.2)
Required dialysis [*n* (%)]	3 (3.5)
Pre- and Post-treatment Creatinine and CrCl	
Pre-treatment creatinine (in µmol/L) (median, IQR, range)	77, 52 – 144.5, 30 – 757
Pre-treatment CrCl (in ml/min) (median, IQR, range)	96, 54.8 – 151.5, 15 – 355
Post-treatment creatinine (in µmol/L) (median, IQR, range)	97.5, 57.5 – 189.5, 26 – 1558
Post-treatment CrCl (in ml/min) (median, IQR, range)	74.5, 36.3 – 123, 8 – 387
Concomitant nephrotoxins used [n (%)]	44 (37.3)

CKD – Chronic kidney disease; RRT – Renal replacement therapy; CrCl – Creatinine clearance; IQR – Interquartile range; KIDGO – Kidney Disease Improving Global Outcomes

$\forall$ A CrCl of $\leq$ 60ml/min based on Cockcroft-Gault equation was considered abnormal.[14]

$*$ Chronic kidney disease as part of the KIDGO classification and not yet receiving dialysis.

$\ni$ Chronic kidney disease as part of the KIDGO classification and receiving dialysis.

$\wedge$ In pre-existing renal failure, nephrotoxicity was defined as $\geq$ than 50% increase in the serum creatinine level from baseline value or a decline in renal function that required renal replacement therapy (RRT).[18]

$\#$ In patients with normal renal function, nephrotoxicity was defined as $\geq$ than two-fold increase in serum creatinine from baseline value anytime during colistin treatment.[18]

Table 10. Multivariate Analysis of Variables Associated with Nephrotoxicity		
Variables	**OR (95% CI)**	**p-value**
***Staphylococcus aureus* cultured**	21.841 (1.589 – 298.419)	0.021
14-day-mortality	67.955 (3.317 – 1392.070)	0.006
Treatment failure	25.136 (3.568 – 177.095)	0.001
Nephrotoxins used	2.276 (0.750 – 6.906)	0.146
In hospital mortality	0.109 (0.007 – 1.828)	0.124
OR – Odds ratio; CI – Confidence interval		

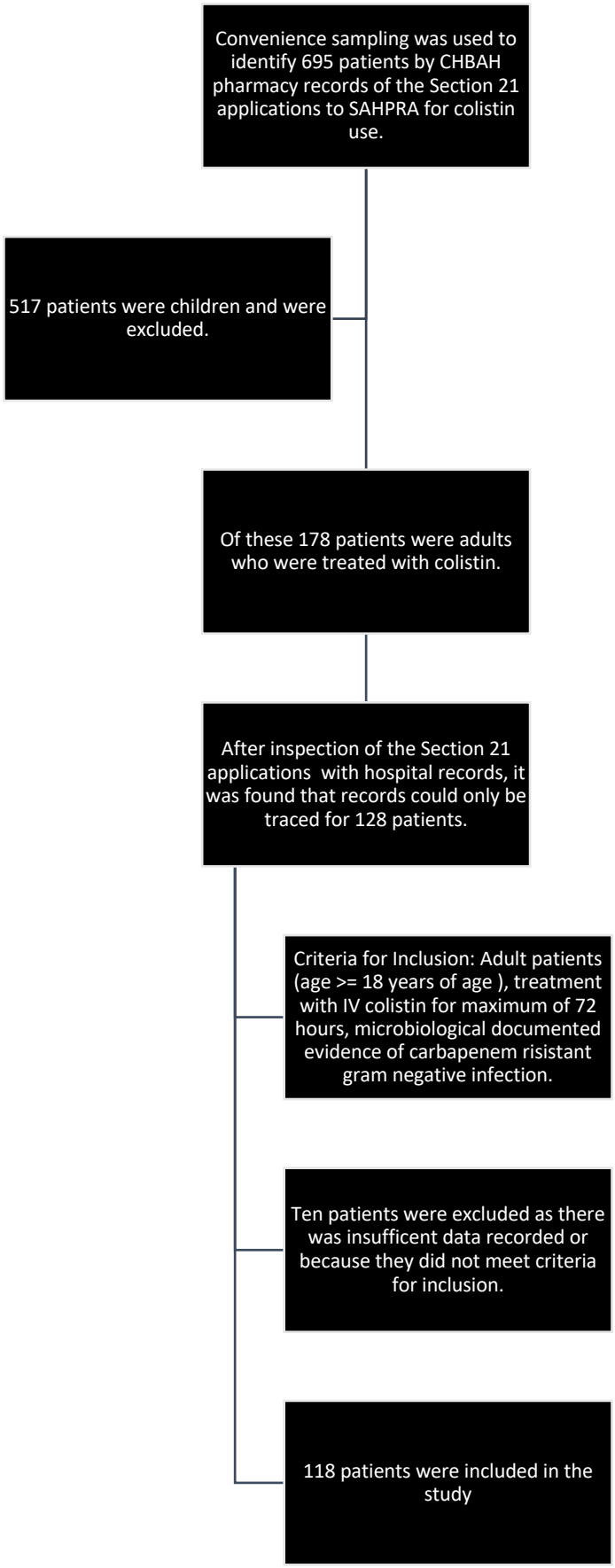


Figure 1 – Figure Showing Selection and Screening of Patients

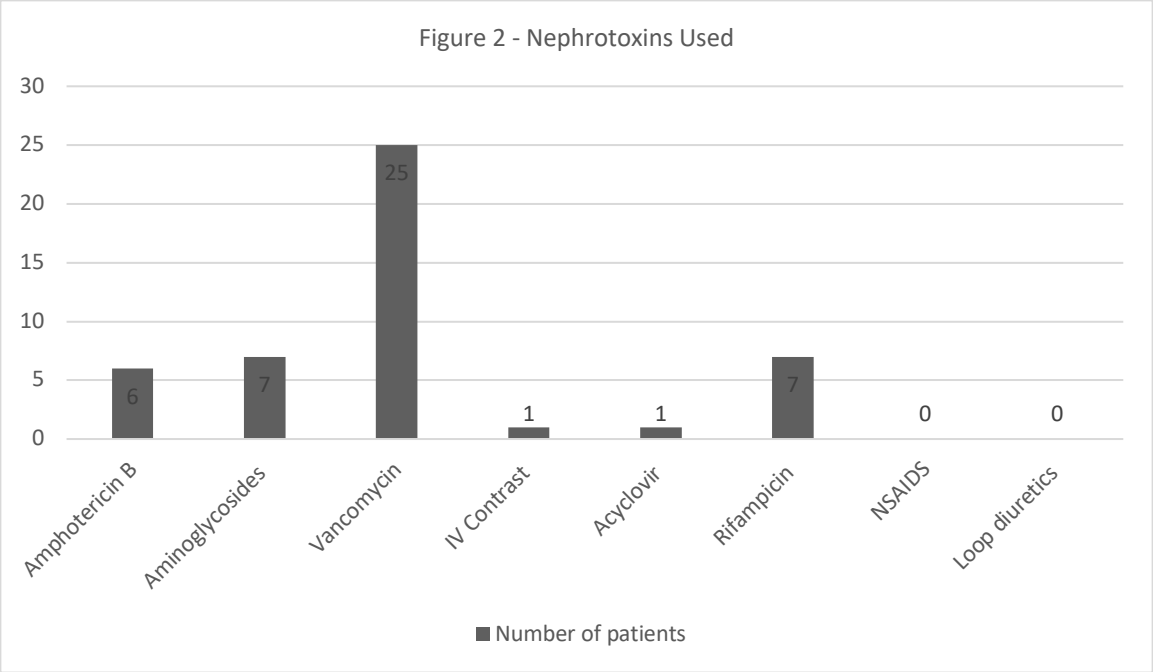


Figure 2 - Nephrotoxins Used n (%) - Amphotericin B – 6 (15.0), Aminoglycosides – 7 (17.5), Vancomycin – 25 (56.8), NSAIDS - 0, (0), IV Contrast – 1 (2.5), Acyclovir – 1 (2.5), Loop diuretics – 0 (0), Rifampicin – 7 (15.9)

References

1 Kanj SS, Kanafani ZA. Current Concepts in Antimicrobial Therapy Against Resistant Gram-Negative Organisms: Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae, Carbapenem-Resistant Enterobacteriaceae, and Multidrug-Resistant Pseudomonas aeruginosa. Mayo Clinic Proc. 2011; 86(3):250-259.

2 Sarkar S, Evelyn R, DeSantis H, Kuper J. Resurgence of colistin use. Am J Health-syst Pharm. 2007;64:2462-6.

3 O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Analysis and Policy Observatory [Internet]. 2016 [cited 11/1/2020].
Available from: https://apo.org.au/node/63983

4 Polymyxins: An overview. Wolters Kluwer [Internet]. 2020 [cited 1/2/2020].
Available from: https://www.uptodate.com/contents/polymyxins-an-overview

5 Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005;40(9):1333-41.

6 Michalopoulos A, Falagas ME. Colistin and polymyxin B in critical care. Crit Care Clin. 2008;24(2):377-91.

7 Laxminarayan R, Van Boeckel T, Frost I, Kariuki S, Khan EA, Limmathurotsakul D, et al. The Lancet Infectious Diseases Commission on antimicrobial resistance: 6 years later. Lancet Infect dis. 2020;20(4):e51-e60.

8 Executive summary: the selection and use of essential medicines. Report of the 21st WHO Expert Committee on the Selection and Use of Essential Medicines. WHO [Internet]. 2017 [cited 20/3/2020].
Available from:
https://apps.who.int/iris/bitstream/handle/10665/273826/EML-20-eng.pdf?ua=1

9 Kift EV, Maartens G, Bamford C. Systematic review of the evidence for rational dosing of colistin. S Afr Med J. 2014;104(3):183-6.

10 Nation RL, Li J, Cars O, Couet W, Dudley MN, Kaye KS, et al. Framework for optimisation of the clinical use of colistin and polymyxin B: the Prato polymyxin consensus. Lancet Infect Dis. 2015;15(2):225-34.

11 Labuschagne Q, Schellacka N, Gousa A, Bronkhorsta E, Schellacka G, Tondera L et al. COLISTIN: adult and paediatric guideline for South Africa. South Afr J Infect Dis. 2016;31(1):3–7.

12 Al Ramahi JW, Al Bashaireh A, Salman S. Colistin in Critically Ill Patients: A Critical Review. IAJAA [Internet]. 2014 [cited 11/1/2020];4(2). Available from: http://imed.pub/ojs/index.php/IAJAA/article/view/939

13 Vardakas KZ, Mavroudis AD, Georgiou M, Falagas ME. Intravenous colistin combination antimicrobial treatment vs. monotherapy: a systematic review and meta-analysis. ijantimicag. 2018;51(4):535–547.

14 Gounden R, Bramford C, van Zyl-Smit R, Cohen K, Maartens G. Safety and effectiveness of colistin compared with tobramycin for multi-drug resistant Acinetobacter baumannii infections. BMC Infect Dis. 2009;9:26.

15 Cheng C, Sheng W, Wang J, Chen C, Chang S. Safety and efficacy of intravenous colistin (colistin methanesulphonate) for severe multidrug-resistant Gram-negative bacterial infections. Int J Antimicrob Agents. 2010;35:297-300.

16 Desai K, Kazi M, Ajbani K, Munshi M, Rodriges C, Soman R, Shetty A. Clinical outcomes and safety of colistin in treatment of gram-negative infections: A prospective observational study. Egypt J Crit Care Med. 2016;4(2):67-72.

17 Messina AP, Brink AJ, Richards GA, van Vuuren S. Opportunities to optimize colistin stewardship in hospitalized patients in South Africa: Results of a multisite utilization audit. S Afr Med J. 2018;108(1):28-32.

18 Gibbs A, Reddy T, Dunkle K, Jewkes R. HIV-Prevalence in South Africa by settlement type: A repeat population-based cross-sectional analysis of men and women. PLoS One [Internet]. 2020 [cited 20/4/2021];17;15(3) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7077822/

19 ACT/DDD Index 2020. WHO [Internet]. 2019 [cited 20/8/2020]. Available from: http://www.whocc.no/atc_ddd_index/?code=J01XB0

20 Cho YS, Yim H, Yang HT, Hur J, Chun W, Kim JH, Lee BC, Seo DK, Kim D. Use of parenteral colistin for the treatment of multi-resistant Gram-negative organisms in major burn patients in South Korea. Infection. 2012;40(1):27-33

21 Gu Z, Han Y, Meng T, Zhao S, Gao C, Huang W. Risk Factors and Clinical Outcomes for Patients with Acinetobacter baumannii Bacteremia. Medicine (Baltimore). 2016;95(9):e2943.

22 Dimitriades K, Morrow BM, Jeena P. A retrospective study on the effects of colistin therapy in children with multidrug-resistant Gram-negative bacterial pathogens: impact of HIV status on outcome. Arch dis child. 2014;99(3):262-266.

23 Wertheim H, Van Nguyen K, Hara GL, Gelband H, Laxminarayan R, Mouton J, Cars O. Global survey of polymyxin use: A call for international guidelines. JCAR. 2013;1(3):131-134.

24 Cheng IL, Chen YH, Lai CC, Tang HJ. Intravenous Colistin Monotherapy versus Combination Therapy against Carbapenem-Resistant Gram-Negative Bacteria Infections: Meta-Analysis of Randomized Controlled Trials. J. Clin. Med. 2018;7:208.

25 Ozel AS, Ergönül Ö, Korten V. Colistin nephrotoxicity in critically ill patients after implementation of a new dosing strategy. J Infect Dev Ctries. 2019;13(10):877-885.

26 Lopez JA. Jorge S. The RIFLE and AKIN classifications for acute kidney injury: a critical and comprehensive review. Clin Kidney J. 2013;6: 8–14

27 Cai Y, Lee W & Kwa AL. Polymyxin B versus colistin: an update. Expert Rev. Anti-infect. Ther. 2015;13:12:1481-1497.

28 Colistin vs Polymyxin B. Relias Media. 1/7/2016. [cited 12/11/2020]. Available from: https://www.reliasmedia.com/articles/138130-colistin-vs-polymyxin-b

29 Vardakas KZ, Falagas ME. Colistin versus polymyxin B for the treatment of patients with multidrug-resistant Gram-negative infections: a systematic review and meta-analysis. Int J Antimicrob Agents. 2017;49(2):233-238.

30 Perovic O, Ismail H, Quan V, Bamford C, Nana T, Chibabhai V et. al. Carbapenem-resistant Enterobacteriaceae in patients with bacteraemia at tertiary hospitals in South Africa, 2015 to 2018. Eur J Clin Microbiol Infect Dis. 2020 Jul;39(7):1287-1294.

31 Boyles TH, Whitelaw A, Bamford C et al. Antibiotic stewardship ward rounds and a dedicated prescription chart reduce antibiotic consumption and pharmacy costs without affecting inpatient mortality or re-admission rates. PLoS One [Internet]. 2013;8 [cited 20/4/2021]. Available from: https://pubmed.ncbi.nlm.nih.gov/24348995/

32 EM Guidance: Empiric Antibiotic Choices at CHBAH. Chris Hani Baragwanath Hospital & University of the Witwatersrand. [Internet]. 2019 [cited 20/4/2021]. Available from: https://emguidance.com/discover

33 Sepsis syndromes in adults: Epidemiology, definitions, clinical presentation, diagnosis, and prognosis. Wolters Kluwer [Internet]. 2019 [cited 11/2/19]. Available from:https://www.uptodate.com/contents/sepsis-syndromes-in-adults-epidemiology-definitions-clinical-presentation-diagnosis-and-prognosis

34 Osei Sekyere J. Current State of Resistance to Antibiotics of Last-Resort in South Africa: A Review from a Public Health Perspective. Front Public Health. 2016;4:209.

35 Zak-Doron Y, Benattar D, Pfeffer I, Daikos GL, Skiada A, Antoniadou A et. al. The Association Between Empirical Antibiotic Treatment and Mortality in Severe Infections Caused by Carbapenem-resistant Gram-negative Bacteria: A Prospective Study. Clin Infect Dis. 2018;67(12):1815-1823.

CHAPTER 3 – APPENDICES

Appendix A – CMS Dosing Guidelines for the Treatment of MDR-GNB	
Adult Guideline 1 (2014)♣	
Loading Dose	
Critically ill or severe sepsis	9-12 MU*
Maintenance Doses	
eGFR > 60 ml/min	4.5 MU 12-hourly
eGFR 30-60 ml/min	3 MU 12-hourly
eGFR 10-30 ml/min	2 MU 12-hourly
eGFR < 10 ml/min	1 MU 12-hourly
Intermittent haemodialysis	1 MU 12-hourly plus supplemental dose of 1 MU after each episode of dialysis
Continuous renal replacement	4.5 MU 12-hourly
Adult Guideline 2 (2016)♦	
Normal renal function	Loading dose 12 MU, then 3 MU 8-hourly or 4.5 MU 12-hourly
Renal Impairment	
CrCl 40-60 ml/min	2 MU 12-hourly
CrCl 10-40 ml/min	2 MU 24-hourly
CrCl < 10 ml/min	1.5 MU 36-hourly
Renal Replacement Therapy (RRT)	
Haemodialysis	As per CrCl, with an additional 2 MU after dialysis
CVVHD	Dosing as for normal renal function

CMS – Colistimethate sodium; MDR-GNB – Multi-drug resistant gram-negative bacteria; e-GFR – Estimated glomerular filtration rate; CrCl – Creatinine clearance; MU – Million units; CVVHD – Chronic veno-venous haemodialysis

♣ Kift EV, Maartens G, Bamford C. Systematic review of the evidence for rational dosing of colistin. S Afr Med J. 2014;104(3):183-6.

* Loading dose calculated according to ideal body weight; 12 MU for 70kg individual and 9 MU for a 55kg individual

♦ Q Labuschagne, N Schellacka, A Gousa, E Bronkhorsta, G Schellacka, L Tondera et al. COLISTIN: adult and paediatric guideline for South Africa. South Afr J Infect Dis. 2016. 31(1):3–7.

Appendix B - Different Formulations of Intravenous Colistin Available			
Dosing/Labelling Convention	**United States Product**	**European Product**	**Product accessed in South Africa**
Milligrams (mg) of CBA	**<u>Sold in vials of 150 mg of CBA per vial.</u>** Dosing in patients with normal renal function is 2.5-5 mg/kg of CBA per day divided into 2 to 4 equal doses.	One vial (1 MU) of CMS is equivalent to 30mg of CBA.	One vial (1 MU) of CMS is equivalent to 30mg of CBA.
Million units (MU) of CMS	Equivalent to 5 million MU of CMS.	**<u>Sold in vials of 1 MU or 2 MU of CMS.</u>**	**<u>Available in vials of 1 MU of CMS.</u>**

CMS – Colistimethate sodium; CBA – Colistin base activity

* Loading doses vary by ideal body weight – see *Appendix A*

R14/49 Micky Sunnyraj

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170835

NAME: (Principal Investigator)	Micky Sunnyraj
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Safety and Efficacy of Colistin Use - A South African Tertiary Level Experience
DATE CONSIDERED:	25/08/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof A. Karsteadt and Prof C. Menezes
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	11/12/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review August and will therefore be due in the month of August each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Micky Sunnyraj

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C – Human Research and Ethics Committee Approval

APPENDIX D – DATA COLLECTION FORM SAFETY AND EFFICACY OF COLISTIN USE – A SOUTH AFRICAN TERTIARY LEVEL EXPERIENCE					
UNIQUE PARTICIPANT IDENTIFICATION NUMBER:					
DEMOGRAPHIC DATA					
Age:	Gender:		Male		Female
CO-MORBIDITIES					
Renal disease	Yes	No			
On RRT	Yes	No			
Diabetes	Yes	No			
HIV	Yes	No	If yes, CD_4 count:		
			If yes, HIV viral load:		
			If yes, is the patient taking ART?		
Malignancy	Yes	No	If yes:	Solid tumour	Haematological
			If yes, define		
Respiratory disease	Yes	No	If yes, define:		
Hypertensive	Yes	No			
Cardiac disease	Yes	No	If yes, define:		
Neurological disease	Yes	No	If yes, define:		
Previous admission in past three months	Yes	No	If yes, when:		
Use of immunosuppressive agents	Yes	No	If yes, define:		
Intercurrent fungal infection	Yes	No	If yes, define:		
HOSPITALIZATION AND ADMISISON					
Length of hospitalization in days:					
ICU admission	Yes	No	If yes, then APACHE II score on admission to ICU:		
			If yes, then duration of stay in ICU:		
Medical ward patient	Yes	No			
Surgical ward patient	Yes	No			
Gynaecology ward patient	Yes	No			
Burns unit patient	Yes	No			
SITE OF POSITIVE CULTURE OF CARBAPENEM RESISTANT ORGANISM					
Bloodstream	Yes	No	If yes, isolated pathogen and date:		
Sputum/tracheal	Yes	No	If yes, isolated pathogen and date:		
Wound pus swab	Yes	No	If yes, isolated pathogen and date:		
Cerebrospinal fluid	Yes	No	If yes, isolated pathogen and date:		
Arterial/CV catheter tip	Yes	No	If yes, isolated pathogen and date:		
Urine	Yes	No	If yes, isolated pathogen and date:		
Tissue	Yes	No	If yes, isolated pathogen and date:		
Other/Unspecified	Yes	No	If yes, isolated pathogen and date:		
DETAILS OF TREATMENT WITH COLISTIN					
Section 21 of MCC[§]	Yes	No	DDD[*]:		
			Duration of treatment:		
			Date started:		
			Duration of non-treatment:		
			Number of doses given:		
Were any doses missed?			If yes, then reason for missed doses:		
What was the weight documented?					
Combination therapy	Yes	No	If yes, specify antibiotic:		
Was a loading dose given	Yes	No	If yes, define:		
Was loading dose adjusted for renal dysfunction	Yes	No	If yes, define:		
Was a maintenance dose given?	Yes	No	If yes, define:		
Appropriately renally adjusted	Yes	No			
Is the patient on dialysis?	Yes	No			

CLINICAL CLEARANCE			
Initial signs of infection	Yes	No	If yes, which of the following:
			Temperature > 38 °C or less than 36 degrees Celsius
			WCC>12x10^9/L or < 4x10^9/L
			Heart rate > 90
			Respiratory rate > 20 breaths per minute
			Purulent sputum/tracheal aspirate
			Ionotropic support necessary
			Elevated CRP
			Elevated PCT
Favourable outcome	Yes	No	***Complete/partial resolution of signs and symptoms and biomarkers without maintenance therapy***
Treatment failure	Yes	No	***Persistence of signs and symptoms and elevated biomarkers or reoccurrence after discontinued colistin***
Death during treatment	Yes	No	***Death or treatment failure are unfavourable outcomes***
Indeterminate outcome	Yes	No	***Inability to assess clinical clearance***
MICROBIOLOGICAL CLEARANCE			
Isolate colistin susceptible	Yes	No	
Was culture polymicrobial	Yes	No	
Organisms cultured			
Date of culture			
Follow up culture done	Yes	No	
Was repeat isolate susceptible	Yes	No	
Was repeat culture polymicrobial	Yes	No	
Organisms cultured			
Date of repeat culture			
Eradication from site	Yes	No	If No, was it the same or different pathogen isolated?
Treatment failure	Yes	No	***Persistence of same organism on follow-up culture from same site.***
Super-infection	Yes	No	***Same or different organisms isolated on follow-up cultures from different sites***
NEPHROTOXICITY ASSESSMENT			
Use of other nephrotoxins	Yes	No	If Yes, specify:
Normal baseline function	Yes	No	Baseline creatinine value before treatment:
Dialysis during/after Rx	Yes	No	Maximum creatinine value during treatment:
NEUROTOXICITY ASSESSMENT			
Neuromuscular blockade	Yes	No	If Yes, other possible cause documented:
Seizures	Yes	No	If Yes, other possible cause documented:
Ataxia	Yes	No	If Yes, other possible cause documented:
Diplopia	Yes	No	If Yes, other possible cause documented:
Paraesthesia	Yes	No	If Yes, other possible cause documented:
Use of sedatives	Yes	No	If Yes, other possible cause documented:
Did symptoms resolve	Yes	No	
OTHER SIDE-EFFECTS DURING TREATMENT			
Hypersensitivity reaction	Yes	No	
Other side-effects	Yes	No	If Yes, please specify:
MORTALITY			
Death in hospital	Yes	No	If Yes, date of in-hospital-mortality:
Mortality at	7-day-mortality		14-day-mortality / 28-day-mortality
			If after treatment, how many days after:
SUMMARY OF LABORATORY RESULTS			

INVESTIGATION	BEFORE Rx	DURING Rx/AFTER Rx
White Cell Count		
Haemoglobin concentration		
Platelets		
CRP		
PCT		
Albumin		
Alanine transaminase		
INR		
Creatinine		
CrCl		
eGFR		
Maximal Creatinine during Rx		

RRT – Renal Replacement Therapy; HIV – Human Immunodeficiency Virus; CD_4 – Cluster of differentiation 4; ICU – Intensive care unit; APACHE II – Acute Physiology and Chronic Health Evaluation Score II; CV – Central venous; MCC – Medicines Control Council; DDD – Defined daily dose; °C – degrees Celsius; WCC – White cell count; CRP – C-reactive protein; PCT – Procalcitonin; Rx – Treatment

§ Was the application to the Medicines Control Council application form for colistin use completed?

* Defined Daily Dose = No. of items issued X amount in each vial/WHO DDD measure (WHO DDD measure for parenteral use of colistin is 3 MU)

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Appendix E – TURNITIN Originality Report