...vity of colistin against *Acinetobacter baumannii* in patients with normal versus impaired renal function

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This research report is based on the original work performed by Dr JM Wojno. Neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. This work has not been published prior to registration for this degree.

Outline

Research proposal

Title:

Serum bactericidal activity of colistin against *Acinetobacter baumannii* in patients with normal versus impaired renal function.

Aims:

The aim of this study will be to determine whether the adjusted doses of colistin methanesulphonate recommended for treatment of patients with renal impairment will result in the same or similar serum bactericidal titres as those achieved using the doses recommended for patients with normal renal function. The hypothesis is that the use of ôadjusted dosesô of colistin methanesulphonate result in significantly reduced serum bactericidal activity.

Background:

There has been a worldwide increase in the number of infections caused by multi-drug resistant gram negative bacteria such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumanii.* This has led to an increased use of the polymyxins such as colistin methanesulphonate. These are often the only effective antibiotics available.

There are currently no standardized dosing regimens for colistin in critically ill patients with renal impairment requiring dialysis (9). Current dosing in patients with various degrees of renal impairment are not supported by adequate evidence. Anecdotal observations of therapeutic failure in patients with renal impairment, have led to a concern that patients are being under-dosed. In contrast, clinical experience shows that patients without renal failure who have *Acinetobacter baumanii* infections respond to the maximum recommended doses of colistin methanesulphonate (Sanofi-Aventis product information for Coly-Mycin® M, 1996). This study undertakes to determine whether current dosage recommendations in renal failure are sub-optimal by comparing *in-vitro* serum bactericidal titres of patients receiving standard versus renally adjusted doses of colistin methanesulphonate.

Colistin has been available for use since the early 1960ôs but fell out of favour due to concerns regarding toxicity. Interest in colistin has resurged in the past ten years due to problems with pan drug-resistant bacteria as well as new encouraging information about its toxicity profile (4). The main toxicities described with parenteral use include nephrotoxicity and neurotoxicity which may be avoided if correct doses are used. These effects appear to be temporary and subside with a reduction in the dose or discontinuation of the drug (5, 12). Recent studies have revealed that colistin is not as nephrotoxic as previously believed and actually has a more favorable profile in terms of nephrotoxicity than the aminoglycosides (9). Dosing guidelines have not been adequately evaluated in patients with renal insufficiency and critically ill patients with severe co-

of information regarding the appropriate dosing regimens odialysis or continuous renal replacement therapy (9). The Coly-Mycin® product information from Sanofi-Aventis has guidelines on dosing in renal failure based on studies by Kucers and Bennet published in a textbook published in 1987. A data search failed to reveal any convincing new information.

Colistin:
Colistin is a polymyxin antibiotic consisting of two components: colistin A (polymyxin E1) and colistin B (polymyxin E2). Two different pharmacological formulations of colistin are available commercially. The first, colistin sulphate, is for oral and topical use. The second, less toxic form, is sodium colistimethate (colistimethate) which is also known as colistin methanesulphonate (6, 7). The terms colistin and colistimethate are not interchangeable. Colistimethate is chemically prepared from colistin. Colistimethate is not stable *in-vitro* or *in-vivo*, and is hydrolyzed when in aqueous solution at 37 degrees, e.g., in plasma or broth, to a series of derivatives plus colistin, which has increased antibacterial activity (7, 9).

Pharmacokinetics of colistin:
The rate of hydrolysis depends on physical conditions such as pH and temperature (10). A recent pharmacokinetic study involving cystic fibrosis patients showed the half-life of colistimethate to be 2.1hrs, and that of colistin to be 4.2 hrs (10). In a patient undergoing continuous venovenous haemodialysis (CVVHD), conversion of colistimethate to colistin was rapid and the half-lives of colistimethate and colistin were 6.8 and 7.5hrs respectively (15). The protein binding capacity of colistin is reported as being low. A study in rats found that protein binding was about 55% in rat plasma (14). Peak serum levels after intravenous administration occur within 10min. Higher peak levels occur in patients with renal failure (10).

Pharmacodynamics of colistin:
Colistin has rapid bactericidal activity, and is bactericidal in a concentration-dependent manner (7, 11). Studies on the post antibiotic effect (PAE) of colistin have shown some concerning results indicating that even at high MICôs there may be hetero-resistant sub-populations in a number of the strains studied. The strains also showed unfavorable, unexplainable negative PAEôs (11) suggesting that mono-therapy or extended-interval dosage regimens (e.g., 24 hours) may not be optimal (11).

Dosing of colistin:
The dose of colistin methanesulphonate (Coly-Mycin®) is expressed in international units (IU). One ampoule contains 80mg which is equivalent to 1 million IU. Recent anecdotal treatment failures in many institutions have resulted in an increase of colistin doses in all categories of renal function. This has however not yet been validated (Table 1). The concern with this increase in dosage is a concomitant increase in side effects particularly nephrotoxicity as reported in a study using similarly higher dose regimens (12). In patients with renal failure it is possible that failure of colistin therapy could be due to inadequate drug levels in patients adjusted doses.

The Serum Bactericidal Test (SBT):
This is an *in-vitro* test that attempts to measure the *in-vivo* activity of an antimicrobial agent. The highest titre (greatest dilution of the patientôs serum) that exerts a bactericidal effect on the

This method of testing incorporates the properties of the antimicrobial agent in question (1).

The clinical relevance of SBT testing is sometimes questioned. A study performed at the National Taiwan University Hospital undertook to determine the predictive value of the SBT for mortality in patients infected with multi-drug-resistant *A.baumannii* (11). Patients were treated with various antibiotics/antibiotic combinations including carbapenems, ciprofloxacin, aminoglycosides and sulbactam. Colistin was not used in this study. The authors showed that peak serum bactericidal titre negatively correlated with mortality rate (correlation coefficient -0.43). The survival rate in patients with peak SBT titres of Ó16, Ó8, Ò4 was 100%, 87.5% and 42.4% respectively (p=0.001). Given this strong association, we believe that it will be valid and clinically relevant to compare SBTôs in patients on standard and adjusted doses of colistin in order to determine the efficacy of adjusted renal dosage (11).

Study population:

Inclusion criteria:
All patients commenced on colistin treatment for proven or suspected *Acinetobacter baumannii* infection will prospectively be enrolled. Patients will be recruited from the medical and surgical Intensive Care Units at Groote Schuur Hospital in Cape Town, South Africa. These are typically patients who have an *A. baumannii* isolated from any body site that suggests infection and requires treatment, e.g., ventilator associated pneumonia. It is well known that culture of this organism from many sites may represent colonization rather than infection. Thus only patients who have an infection for which therapy is clinically indicated will be included in the study. This decision will be made by the attending physician. The reason for admission and diagnosis will not impact on inclusion.

Exclusion criteria:

1. Patients who have lipaemic or haemolysed serum. This could affect the interpretation of SBTôs, these being determined according to visual turbidity.
2. Patients who decline consent to participation.

There will be two study groups. Firstly, patients without renal impairment, i.e., normal creatinine clearance. This will be defined as a creatinine clearance of > 90 ml/min for the purposes of this study (Group 1). These patients will be on the highest dose of colistin recommended for normal renal functions as stated in Table 1. Secondly, patients with impaired renal function on adjusted doses of colistin (Group 2).

Materials and Methods:

Sample Collection:
Colistin will be commenced in enrolled patients according to the recommended dosing regimen (highest dose of Coly-Mycin®) at Groote Schuur Hospital as stated in Table 1. Initial blood samples will be taken 3 days after the initial dose is given to allow steady-state to be reached (7).

lebotomist, and a minimum of 1mL of blood will be

Group 1: doses are given every 8 hours. Thus blood will be taken at 30 minutes, 60 minutes, 90 minutes (near the peak level), then at 4 hours and 8 hours (trough level before the next dose is given).
Group 2: dose frequency varies according to degree of renal impairment. Blood will be taken at 30 minutes ,60 minutes and 90 minutes and then will be drawn every 4 hours until the next dose is due i.e., at 4 hours, 8 hours and 12 hours (trough level) if the dose frequency is 12 hourly. Degree of renal impairment, dose adjustment and type of dialysis, if utilized, will be documented in all cases.

Following collection, the blood samples will be immediately placed on ice (to terminate the conversion of colistimethate to colistin) and taken directly to the laboratory, where serum will be separated from the red blood cells in a refrigerated centrifuge and subsequently frozen at -70 C. It has been shown that colistin is stable when frozen (8).

Table 1. Dosing Schedule

	Highest Doses recommended for Coly-Mycin® (16)	Doses recommended by Sanford Guide to Antimicrobial therapy 2008 (17)
Cr Clearance ml/min	**Dose**	**Dose**
Normal renal function:	3 million U 8 hourly	1-2 million U 8 hourly
50-90	3 million U 12 hourly	2 million U 12 hourly
10-50	3 million U daily	2 million U daily
<10	3 million U 36 hourly	2 million U 36 hourly
Anuric	1.5 million U after each episode of dialysis	1 million U after dialysis
CVVHD	3 million units 12 hourly (15)	2-3 million 48 hourly (9)

Laboratory Materials:

- *Sterile, plastic, flat-bottomed microplate 96 well trays*
- *100ul pipettes*
- *10ul pipettes*
- *Pipette tips*
- *Eppendorf tubes 2ml*
- *Mueller-Hinton agar*: needed for SBT determination
- *Acinetobacter baumannii* reference strain*:* The *ATCC BAA-1605* strain available, which is sensitive to amikacin and tobramycin, will not be used as amikacin is widely prescribed and may be a confounder. A strain from the clinical laboratory will be used instead , this

s tested except colistin and tobramycin. In no clinical
ons be used together. The degree of resistance will be
characterized (by doing Minimum Inhibitory Concentration testing using an Epsilometer E-TEST). This standard strain will be used to ensure that results can be compared to each other with no bias due to variation in strain MIC.

- *Plastic pipettes 3ml*
- *Tripticase soya broth (TSB)*
- *2% blood agar*

Methods:
The samples will be tested individually to prevent premature thawing at room temperature. Testing will be performed in sterile, plastic, flat-bottomed MICROLON ® microplate 96 well trays (Lasec SA, South Africa). The reference strain used will be stored at -70°C in a Microbank^{TM} vial containing cryopreservative fluid.

Inoculum preparation and inoculation of wells:
The CLSI (Clinical and Laboratory Standards Institute) recommends that the final inoculum of reference strain in each test well is 5 x 10^5 cfu/ml. In order to achieve this concentration, preparation of the reference strain is required, as follows.

1. First, the reference strain of *Acinetobacter baumannii* will be subcultured three times on standard blood agar plates to ensure that the organism has optimal growth and metabolic status prior to drug exposure (13).
2. Next, suspensions of the organism with a turbidity equivalent to that of a 1 McFarland standard will be prepared after suspending the colonies into a pre-warmed (35°C) Tripticase Soya Broth (TSB -Greenpoint NHLS Media Laboratory, South Africa). This will then be incubated for 6 hours to ensure log-phase growth.
3. Finally, the reference strain will be diluted to achieve the optimal concentration. As a 1 Mcfarland standard contains approximately 3 x 10^8 cfu/ml, and the final concentration of the reference strain in each well once inoculated should be 5 x 10^5 cfu/ml, a 1:30 dilution of the 1 McFarland standard will be performed by adding 0.1ml of the reference strain suspension to 2.9ml of broth. This will yield an inoculum density of 1 x 10^7 cfu/ml, which will be further diluted 1:20 by adding 5ul of this organism suspension to the 100ul (serum/TSB mixture) in the test well. This will yield a final inoculum of 5 x 10^5 cfu/ml in each well.

For each timed sample from a patient e.g. patient A initial sample, a single row of a microwell plate (MWP) will be used, with one MWP per patient. Firstly, 100uL of TSB will be dispensed into wells 1-2 and 4-10 in a single row. Next, 5uL of ATCC strain will be dispensed into the 1st TSB-containing well, which will serve as the positive growth control. The second well will serve as the negative growth control. Into the 3rd and 4th wells, 100uL of neat serum will be dispensed. Serial doubling dilutions will begin from the 4th well using standard techniques with the final well for every sample being a 1:128 dilution. Next 5uL of the ATCC strain will be added to wells 3 to 10, achieving a final volume of 105uL in all 10 wells. According to the NCCLS document the volume of organism suspension added to the well must not exceed 10% of the total volume of the well as this may affect the dilution (13).

l be repeated for the next timed sample collected from the . Once all the rows have been inoculated for a patient (depending on the number of samples collected per patient), the microwell plate will be incubated in air at 35°C for 18-24 hours.

Reading of results:
Wells will only be read if there is definite turbidity in the positive growth control and no growth in the negative growth control. Twenty microlitres of fluid from every well containing no growth will be sub-cultured onto Mueller-Hinton agar to assess the bactericidal activity of the drug. The plates will be incubated in air at 35°C for 18-24 hours. The dilution that demonstrates 99.9% killing, i.e., 20ul of the 5 X 10^5 cfu/ml dilution should contain fewer than 10 colonies on the plate, is the bactericidal titre.

Analysis and Interpretation of results:
The serum bactericidal activity will be assessed against a known (reference strain) of *Acinetobacter baumannii.* Titres of patients receiving dosages of colistin recommended for normal renal function will be compared to those receiving adjusted dosages due to renal impairment.

Subgroup analysis will be performed to evaluate the effect of dialysis on the SBT, however the study will not specifically be powered for this analysis. Additional factors which may influence SBT, such as age and type of illness will be recorded.

Bacterial eradication in vitro cannot predict clinical cure. Many factors play a role including: host factors, post-antibiotic effect and growth-inhibitory effects of sub-MIC concentrations (13). If results show that the titres achieved in the renal failure patient group are much lower than those of the normal renal function group, further studies will be needed to determine what dose increase is required as the risk of drug toxicity is a major concern.

Limitations:

- Inability to directly determine the serum concentration of colistin achieved with a particular dose
- Although SBT predicts bacterial eradication, the clinical relevance of SBT remains controversial (13). *In-vitro* bacterial eradication cannot predict clinical cure.
- Biological assays such as this are unable to differentiate the amount of colistin present in the sample at the time of collection from the colistin formed in vitro by hydrolysis of colistimethate during the microbiological assay (2); however, bias will be minimized by treating all samples in the same manner.
- It has been documented that human serum alone can have a bactericidal effect on certain Acinetobacter *baumannii* strains due to complement mediated lysis (3). This may have a confounding effect.
- Many patients will be on concomitant broad spectrum antibiotics including a carbapenem. Other patients will recently have been on another broad-spectrum antibiotic. Renal failure can affect clearance of these drugs and so there may sometimes be residual antibiotic still present in the patientôs serum, thus the SBT measured may not be solely due to colistin. There can be synergy between colistin and

acter baumannii (14). To minimize this effect a *obacter baumannii* will be used that is resistant to all antimicrobials that may be used locally for the treatment of *Acinetobacter.* This strain will only be sensitive to colistin. This will ensure that there will be minimal effect on the SBT from the other antibiotics if used concomitantly.

Sample size

In order to determine a two-fold difference in SBT level between the two groups (standard dose and renal dose) with 80% power and type 1 error of 0.05, we will require 17 patients per group.

Budget:

The National Health Laboratory Service Research Trust will be approached as a possible source for the funds required.

Items required	**Number of units**	**Cost per unit/box**	**Total Cost**
Laboratory items:	----------	----------	----------
1. Microplate 96 wells flat bottomed	600	R590.00 per box (100ŝ)	R3540.00
2. Mueller-Hinton Agar	600	R3.00 per agar plate	R1800.00
3. Pipette tips FILTER	9000	R950.00 per 1000 tips	R8550.00
4. 1000ul pipettes	2	+/- R4000 each	R8000.00
5. 100ul pipettes	2	+/- R3000 each	R6000.00
6. 10ul pipettes	2	+/- R3000 each	R6000.00
7. A.baumannii strain and transport	1	R1500.00	R1500.00
8. Safelock Eppendorf 1.5ml	500	R500.00	R500.00
9. CAMHB (cation-adjusted Mueller-Hinton broth)	1000ml	R80.00 for 200ml	R400.00
10. 2% blood Agar	600	R3.50 per agar plate	R2100.00
11. Plastic pipette 3ml	1000	R75.00 per packet (500ŝ)	R150.00
Collection items for ward:	----------	----------	----------
12. SST Gel tubes 5ml	300	R100.00 per box (100ŝ)	R300.00
13. Vac needles black	300	R75.00 per box (100ŝ)	R225.00
14. 5ml syringes	300	R55.00 per box (100ŝ)	R165.00

		R50.00 per box (100ôs)	R150.00
16. Safelok blood collection (butterfly)	300	R125.00 per box of 50	R750.00
17. Micro pore tape	30	R10.00	R300.00
18. ETEST strips for : amikacin, tobramycin, gentamicin, ceftazidime, cefepime, co-trimoxazole, ciprofloxacin meropenem, imipenem	2 for each antibiotic	R1050 for a Box of 30 ï a box is needed for each antibiotic	R9450.00

Stationery required: R3000.00
TOTAL: R52 880.00

Ethical consideration:

All blood samples once drawn and sent to the laboratory will be given a laboratory number and only this will be used for sample identification. Patient names and/or hospital numbers will not be mentioned in the study write-up. There will thus be no breach of patient confidentiality.

Treatment with colistin will be commenced at the discretion of the attending physician. Reasons to institute therapy will include proven *Acinetobacter baumannii* infections as well as empiric choices in patients who are developing signs and symptoms of infection and are known to be colonized with the organism.

In terms of patient discomfort whilst drawing blood samples, ICU patients often require blood tests to be done on a daily basis. It will be discussed with staff that other blood tests that need to be done can be done at the same time as the samples required as to minimize patient discomfort. If repeated 4 hourly specimens are required patients will receive a port that will be present for the duration of the blood collection to minimize repeated venipuncture.

Consent may be difficult to obtain from the patients themselves as often the patient is unable to give consent and family members may not be present at the time the decision is made to commence therapy, e.g., sudden deterioration in patientsô condition due to sepsis. Although every effort will be made to get consent, this may be problematic as therapy is often started as an emergency intervention. Every patient who is started on colistin therapy has to have a signed section 21 clearance form from the Medicines Control Council as colistin is not registered for use in South Africa. The Medicines Control Council will be informed of this particular study. The results of this study will not have an impact on patient treatment as the patients own isolate has not been used and one thus cannot individualize dose adjustments. Future dose adjustments may be affected in other patients.

Future prospects:

- If one were to use the patientsôown isolate and not a reference strain, one could use the SBT as a prognostic indicator as it correlates directly with mortality and could be used as an indicator of the need to possibly modify treatment dose in that particular patient. The patientôs own *A.baumannii* isolate will be collected and stored for possible further expansion of this study in the future.
- Collaborators in the Department of Pharmacology will explore the possibility of establishing an assay for direct analysis of colistin and its metabolite in stored samples from this study.

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Literature review

Objectives of literature review:

- To research the pharmacology of colistin (polymyxin E) and related polymyxins with focus on pharmacokinetics, pharmacodynamics
- To review studies performed using colistin in different settings, patient populations and at different doses
- To find a standardized protocol on how to perform a Serum Bactericidal Test (SBT)
- To assess the value of microbiological assays and how they compare to measuring pharmacological levels of colistin
- To determine the gaps in colistin research

Literature search strategy: including exclusion and inclusion criteria:

From September 2008 to December 2009, the search engines PUBMED and Google Scholar were used. The initial search included the keywords: ópharmacology, colistin, polymixin, pharmacodynamics, pharmacokineticsô. Articles in languages other than English were excluded from the search. Other literature included textbooks found at the University of Cape Town Library and the Pharmacology Department at Groote Schuur Hospital in Cape Town.

Finding existing information on the pharmacology of colistin was necessary to prevent repeating previous studies and to inform the study design. The initial selection strategy was broad, in order to extract as much information as possible on polymyxins, in view of the paucity of pharmacological data on colistin itself. Many of the initial studies on colistin cited polymyxin B not the colistin preparation used currently, polymyxin E, which differs by a single amino acid. Moreover it included studies in animals as well as those in cystic fibrosis patients, in whom colistin was initially used. This was in contrast to the study population, however, it provided useful background to the subject. Knowledge of the antimicrobial spectrum, chemical composition, stability at various temperatures and states e.g., in solution and in serum was required. Other necessary parameters included peak levels, volume of distribution, elimination half-life as well as information on the correlates of efficacy. Knowledge on the activity of the drug in different settings, patient populations, at different doses was crucial in order to formulate a research question as well as an explanation of the findings in the population subgroups studied. Previous studies that showed the effects of renal failure/dialysis on colistin levels and action/effect were searched for. Sampling required knowledge of the half-life of colistin in order to work out when steady-state of the drug is reached as well as information on the time after administration that the drug reaches a peak level.

One of the search strategies used was searching by author. This may, however, be limited by the authorôs interests and is possibly prone to bias.

Once the relevant articles had been retrieved by the initial broad search, the search was refined to include studies in critically ill patients, studies in patients with renal impairment as well as

earch yielded information on all the colistin preparations

Clarity on various pharmacological and clinical concepts such as SLED (Sustained Low Efficiency Dialysis) was required.

As the SBT is not performed routinely in a clinical laboratory and the results have not been correlated with clinical efficacy, a study that could prove its clinical utility, usefulness and relevance was needed. A protocol for the Serum Bactericidal Test (SBT) was found in the NCCLS (National Committee for Clinical Laboratory Standards) document as no updated CLSI (Clinical and Laboratory Standards Institute) document was available. This was used to guide accurate and correct performance of the SBT. Information on serum dilutions, materials required, incubation temperatures and durations was searched for in this document to guide protocol steps and methodology (18), which was a variation of the typical SBT. In order to gain a broad perspective on serum bactericidal/ microbiological assays, studies where these tests had previously been performed using other drugs were helpful. This contributed to protocol preparation and provided insight into the interpretation of the SBT in different settings.

The next objective was to find an *Acinetobacter baumannii* strain which met the study requirements. Strains identified in the literature did not contain the resistance patterns that were required. The ATCC Bacteriology Collection also did not have such a strain. The strain chosen was isolated from a sputum sample of a patient admitted to the Respiratory ICU at Groote Schuur Hospital. Information on how to prepare, culture and handle the organism was found in the SBT protocol in the NCCLS document (18).

It was important to determine patient and assay factors that influence the accuracy of the SBT, as well as the advantages and limitations of the microbiological assay compared with alternatives such as pharmacological assays.

Using existing knowledge, a list of potential confounders was drawn up, and a literature search on ñSBTò and each confounder using the advanced search field in PUBMED was performed. With further research and reading, several new possible confounders were identified, which expanded the original list. The protocol was adjusted according to new findings e.g., exclusions were included such as lipaemic/haemolyzed blood that may affect interpretation of the SBT (18).

It became apparent that the use of concurrent antibiotics had to be considered, as this may cause synergism or possible antagonism with colistin. Additional metabolic and chemical/physiological factors that could influence the results were explored such as gender differences, age and volume of distribution of colistin.

Quality criteria: appraisal of studies:

To evaluate/rate the strength of evidence and to critically review the quality of research studies, a guide was required (26). The general approach to assessing research studies, articles and available literature required fine tuning. This was discovered when initial literature searches located thousands of articles.

n adequate study design, selection of subjects, objective equate statistical power to detect results. Research should not be judged solely on whether or not it is published in a leading journal. The authors should provide the reader with enough and adequate details in the ñMaterials and Methodsò in order to replicate the study if required. The study should also contribute to existing knowledge, ask and answer relevant research questions and ideally have comparison groups. Also, it should adequately explain findings and other reasons for the findings. Research papers submitted for peer review are preferable. For a study to be regarded as being of high quality there needs to be evidence that the authors tried to minimize bias in their study designs. There are clearly many factors that need to be assessed before viewing a research article.

The number of studies on the particular topic of interest was limited and the size of the samples was generally small thus affecting statistical power and possible credibility of the results obtained. A quality evidence base often requires consistency i.e., many research studies that show similar findings. Consistency in the particular field of colistin doses and pharmacological data was problematic as there are few studies, particularly in critically ill patients, on colistin. However, in rare cases one study can provide convincing evidence due to the success of the findings in a well-designed and/or adequately powered study. However, the urgency for new information may incorrectly give a study higher than deserved credit as information is urgently awaited and no other data exist. For example: Initial work done on the pharmacokinetics of colistin in dialysis patients was a case report of a single patient on continuous venovenous haemodiafiltration (CVVHD) who was critically ill and required colistin (13). A lot of pharmacological conclusions were drawn from this study and can clearly be erroneous as it was a single anecdotal report of the pharmacokinetics of colistin in this subset of patients.

Knowledge of the half-life of colistin was required in order to work out the creatinine clearance. Many initial studies were performed in cystic fibrosis patients. This is a very specific group of patients with specific pharmacodynamic and pharmacokinetic parameters. Since colistin use has now extended beyond cystic fibrosis patients and is now mainly used in Intensive Care Units (ICUôs) and often in patients with co-morbidity who are critically ill, more colistin studies are warranted.

Very little information on the predictive value of the SBT for mortality/outcome of patients infected with multi-drug resistant organisms exists. The study, published in the Journal of Infection, conducted by researchers at the National University of Taiwan is one such study (14). It correlated the titre obtained at peak, after administration of many classes of antibiotics, with mortality. These findings were statistically significant. The study was powered to detect this difference and was a well-designed study, using multi-variate analysis.

Even though a large volume of information exists on colistin there is very little in the way of hard evidence especially concerning the pharmacokinetic and pharmacodynamic properties of the drug. This paucity of established information has had an effect on the strength of evidence that supports the current dosing recommendations. A lot of the current information is based on studies performed in the late seventies and early eighties. As a last resort many clinicians are basing and justifying their use of higher and higher doses on very few studies and more clinical experience and anecdotal reports. The most prominent finding when searching this ñoldò

rs referred to one another, leading to difficulty in finding

Much of the contemporary research is being conducted by a relatively small number of researchers. The positive aspect to this i.e., research produced by experts in the field is offset by the potential for the research strategy to be biased and unidirectional. The concept of bibliometric analysis applies here. This occurs when work done by a group of researchers is referred to by other authors when citing the research on that particular topic. Whilst the apparent importance of that research is enhanced there may be a degree of bias, and frequent references to a particular finding or study do not necessarily imply they are of high quality.

The cited studies clearly show the discrepancies between the various findings proving that it was prudent to extend the estimation of the half-life due to the knowledge that in cystic fibrosis patients it is frequently shorter.

Summary or interpretation of the literature:

Current literature highlights the increase in multi-drug resistant isolates such as *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* (8, 12, 21). In order to overcome this problem efforts have been made to find alternative treatment strategies and/or improve current knowledge as well as use existing medication to its full potential. This means either looking back at ñforgottenò drugs such as the polymyxins and understanding how to use them optimally, or discovery of new drugs such as tigecycline that offer alternative antimicrobial options. With the limited availability of alternatives there has also been a focus on combination therapy and the synergy that it can provide.

Summary of the Drug, the Bug and the Test:
Colistin: Colistin (polymyxin E) is a polymyxin antibiotic derived from various species of *Paenibacillus polymyxa*. It consists of two components: colistin A (polymyxin E1) and colistin B (polymyxin E2) (9). Colistin is administered as colistimethate sodium (colistimethate) which is also known as colistin methanesulphonate (8, 9, 17). Colistin methanesulphonate (CMS) is known to be unstable in aqueous solution as it hydrolyzes into a mixture of sulphomethyl derivatives plus colistin (12). Colistin is more biologically active than CMS and due to the instability of CMS it is uncertain how much is present in the administered product (3, 7, 9, 12). *In-vitro* studies have shown that colistin is bactericidal in a concentration-dependent manner and that a modest post-antibiotic effect occurs when high concentrations are used (17).

While previous concerns regarding toxicity of colistin prompted clinicians to use alternative therapies, newer studies, as well as a lack of alternatives, have caused resurgence in the clinical use of colistin (6, 25). The most frequent adverse effects of polymyxins include nephrotoxicity and neurotoxicity, which are reversible upon cessation of the antibiotic (17). The polymyxins are known to be bactericidal. They disrupt the outer membrane of the organism causing cell lysis and death (17). The main indication for the use of colistin is the treatment of multidrug-resistant gram-negative bacteria that have reduced susceptibility to many other antimicrobial agents. The polymyxins were initially used in cystic fibrosis patients but are being required more regularly in critically ill patients in the Intensive Care Unit (ICU) setting.

rmacokinetics and pharmacodynamics stems from research in animals as well as in cystic fibrosis patients. These data may be erroneous when applied to other patient groups as differences exist, such as shorter half-lives of drugs in cystic fibrosis patients (12, 13). Various small studies have also been conducted in different settings, with different patient profiles, using different study designs. Many of these studies have shown discrepant results thus causing confusion in the doses recommended for various patient groups. This is because the pharmacokinetic/pharmacodynamic (PK/PD) parameters have not yet been clearly defined. The results of the half-life of CMS and colistin for example, have shown major discrepancies. A study involving cystic fibrosis patients showed the half-life of CMS to be 2.1 hours, and that of colistin to be 4.2 hours (22), while another study in this group of patients found that the half-life of CMS and colistin was 2.3 hours and 6.4 hours respectively (9). The dose recommendations of the various colistin preparations have been based on such information as well as studies on polymyxin B (7, 17, 23). When comparing the half-life determined previously to a more recent study in critically ill patients by Plachouras et al, it was shown that previous calculations of half-life could have been erroneous. Previous studies showed shorter values due to possible conversion of CMS to colistin before administration of the product. Plachouras et al estimated a typical half-life of colistin in critically ill patients to be 14.4 hours (22).

Few studies have been performed in critically ill patients. Colistin use is increasing in this setting as well as in patients with renal impairment requiring various forms of dialysis. As PK/PD data in this group of patients is lacking, dosage recommendations have been problematic.

Pharmacological levels of CMS and colistin have been assessed in a critically ill patients undergoing CVVHD in a study by Markou et al that found half-lives in critically ill patients at steady-state with varying renal function to be between 3.8-9.5 hours (15). As CMS is cleared by the renal route, whereas colistin is cleared by the non-renal route (17), a dosing strategy that maintains the size of the dose but extends the dosage interval has been proposed (13). The study in a single critically ill patient by Li et al showed that both colistin and CMS are removed by CVVHD (13). The authors did, however, state that the dose adjustment used in this patient should have been more modest. The study by Plachouras et al (22) also showed little correlation between colistin kinetics and creatinine clearance, supporting previous suggestions that colistin is cleared by the non-renal route. This study also suggested that current dosing may lead to colistin concentrations below the MIC breakpoints of usual gram-negative organisms after the first few doses of CMS. They suggest that initial doses could be increased without concomitant increase in toxicity. Loading doses as high as 12 million units have been suggested. Markou et al also performed a study in critically ill patients which suggested there was suboptimal dosing due to inadequate maximum concentrations of colistin being reached (15).

Acinetobacter baumanni:
This organism is a gram negative, aerobic non-fermenting bacterium that is a frequent cause of nosocomial infections such as ventilator associated pneumonia (VAP) and bacteraemia, especially in the ICU setting. Globally, there has been a rapid emergence of strains of *Acinetobacter baumannii* that are resistant to many antibiotic classes. This may be due to the fact that the organism is readily able to acquire many new resistance mechanisms, limiting the

Interest in colistin has arisen as it has become one of the some of these pan-drug resistant strains (5, 11, 20, 30). Resistance to the polymyxins is uncommon. Resistance has, however, been reported in certain instances where colistin has been used as a prolonged treatment for multidrug resistant organisms (17). Resistance to colistin can be due to modification of the lipid A component of the outer membrane, proteolytic destruction of the drug, as well as removal of the antibiotic by an efflux pump (1). It is important to consider sub-optimal dosing as a cause of emergence of colistin resistant isolates.

The Serum bactericidal test (SBT):

This microbiological assay is an in vitro test that attempts to measure the *in-vivo* activity of an antimicrobial agent. The highest titre (greatest dilution of the patientôs serum) that exerts a bactericidal effect on the organism is the serum bactericidal titre. This method of testing incorporates the pharmacodynamic and pharmacokinetic properties of the antimicrobial agent in question (2, 26). There are both advantages and disadvantages of microbiological and pharmacological assays. While pharmacological assays measure drug levels using High Performance Liquid Chromatography (HPLC) for instance, the SBT measures the final effect of the drug, which is bacterial death. Both methods need to be correlated with the clinical outcome of the patient for the result to be relevant. This has been found in pharmacological assays of other drugs such as aminoglycosides, where measuring peak levels has been shown to provide a useful therapeutic monitoring tool. Such a measurement has not yet been established for colistin. Pharmacolocical asays are important to determine the PK of various compounds such as CMS and colistin. The microbiological assay is unable to distinguish the colistin formed *in-vitro* (while performing the assay) to that formed *in-vivo*. It has been used in the past to confirm that bacterial eradication has been achieved but this has not been correlated with clinical outcomes (10, 18). The prognostic value and clinical relevance of microbiological assays have been questioned in the past. However, it is thought that a bactericidal effect is crucial when considering patients with deep-seated infections such as endocarditis (18). No previous published attempt has been made to look at SBTôs in patients on colistin. A study conducted at the National Taiwan University showed how the SBT can be used to predict patient outcome (14). This study aimed to determine the predictive value of the SBT for mortality in patients infected with multi-drug-resistant *A.baumannii*. Patients were treated with various antibiotics/antibiotic combinations including carbapenems, ciprofloxacin, aminoglycosides and sulbactam. Colistin was not used in this study. The authors showed that the peak serum bactericidal titre negatively correlated with mortality rate (correlation coefficient -0.43). The survival rate in patients with peak SBT titres of Ó16, Ó8, Ò4 was 100%, 87.5% and 42.4% respectively (p=0.001). Given this strong association, it would be clinically relevant to compare SBTôs in patients on standard and adjusted doses of colistin in order to determine the efficacy of the adjusted renal dose.

Due to the limited options of antibiotics available when treating pandrug-resistant organisms, a number of studies have been performed that assessed combination therapy with colistin for the treatment of *Acinetobacter baumannii* infections. This was done to assess synergy and possibly offer hope of preventing the emergence of resistance. The method of synergy testing used in these studies included checkerboard and time kill methods and used colistin in combination with rifampicin, minocycline, ceftazidime, meropenem, azithromycin, doxycycline, ampicillin-sulbactam or trimethoprim-sulfamethoxazole. Many are limited by patient numbers, but there

he combination of colistin and rifampicin (19, 24, 27).
nation with, colistin have been inconclusive (4, 28, 29, 31). There have also been a few promising combination studies performed in animal models (16, 19).

Identification of gaps and/or needs for further research:

- More information on the other active metabolites of colistimethate.
- Information on the exact effect of dialysis on colistin as well as more data on how the dialysis affects pharmacological levels of colistin. Outcome data stratified according to type of dialysis used (as well as dose administered) is urgently required.
- Generally there are few randomized control studies on the PK/PD parameters of colistin. The pharmacodynamic parameters, such as AUC/MIC and %T/MIC, that are most applicable to colistin need to be defined and correlated with clinical efficacy. This could affect dosing practices, for instance, if dosing with high peak levels once a day would be more beneficial than more frequent dosing.
- More studies are required on the SBT and data on whether it can be used as a tool to assess patient outcome in various population groups.
- Loading doses have frequently been addressed as potential improvements to dosing. No data exist on their value, or their margin of safety or concentration needed.
- More information on synergy and possible combinations of other medication with colistin. It has been shown that resistance to colistin has already emerged. Alternative treatment approaches need to be investigated.
- Assess whether sub-therapeutic dosing leads to the emergence of resistant strains and sub-populations.

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The Manuscript

Serum bactericidal activity of colistin against *Acinetobacter baumannii* in patients with normal versus impaired renal function

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Standardized dosing regimens for colistin in critically ill patients with renal impairment requiring dialysis, are lacking. This is due to the paucity of pharmacokinetic and pharmacodynamic data. Due to the dramatic rise in pan-resistant organisms that test sensitive only to colistin, information regarding its efficacy at the current recommended dosages is crucial. Colistin drug level measurements have recently been attempted but have not, as yet, been confidently correlated with microbiological and clinical outcomes. This study aims to determine microbiological efficacy of colistin at various time points after colistin dose administration by using the bactericidal titre of the patient's serum against a standardized laboratory strain of *Acinetobacter baumannii.* Serum bactericidal titres from patients with renal impairment dosed according to their degree of renal impairment or dialysis requirements were compared to titres achieved in patients with normal renal function receiving a standard dose. Our results show that this microbiological assay, albeit relatively crude, shows a favourable correlation between the titres of the two patient groups throughout most of the dosing interval. The subset of patients on dialysis, however, who were dosed according to dialysis recommendations, tended toward lower titres at certain time points of the dosing interval. The data suggests that patients receiving doses adjusted for renal failure are achieving similar serum levels that could result in successful treatment. Higher doses in patients on dialysis, may however need to be considered.

Introduction

There has been a worldwide increase in the number of infections caused by multi-drug resistant gram negative bacteria such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii.* This has led to an increased use of polymyxins (such as colistin) which are often the only effective antibiotics available (10).

ing regimens for colistin in critically ill patients with
10). Guidelines that are currently in use for dosing at
various degrees of renal impairment are not supported by adequate evidence. Anecdotal observations of therapeutic failure in patients with renal impairment have led to concerns that patients are being underdosed. In contrast, clinical experience shows that patients without renal failure who have *Acinetobacter baumanii* infections respond to the maximum recommended doses of colistin (Sanofi-Aventis product information for Coly-Mycin®, 1996). This study aimed to determine whether current maximum dosage recommendations in renal failure are sub-optimal by comparing serum bactericidal titres of patients receiving normal versus renally adjusted doses of colistin.

Colistin has been available for use since the early 1960ôs but fell out of favour because of concerns regarding toxicity. Interest in colistin has surged in the past 10 years due to problems with pan drug-resistant bacteria as well as new encouraging information about its toxicity profile (4). The main toxicities described with parenteral use include nephrotoxicity and neurotoxicity which may be avoided if correct doses are used. Both of these effects appear to be temporary and subside with a reduction in the dose or discontinuation of the drug (7, 14). New studies have revealed that colistin is not as nephrotoxic as previously believed and actually has a more favorable profile in terms of nephrotoxicity than aminoglycosides (10). A clinical dilemma arises in patients with renal insufficiency and critically ill patients with severe co-morbidities as dosing guidelines have not been adequately evaluated (8). There is also a paucity of information regarding the appropriate dosing regimens for patients requiring haemodialysis or continuous renal replacement therapy (10). The Coly-Mycin® product information from Sanofi-Aventis has guidelines on dosing in renal failure based on studies by Kucers and Bennet published in 1987. A data search failed to reveal any convincing new information.

The antibacterial agent known as colistin is a polymyxin antibiotic consisting of two components: colistin A (polymyxin E1) and colistin B (polymyxin E2). Two different pharmacological formulations of colistin are available commercially. The first, colistin sulphate, is for oral and topical use. The second, less toxic form, is sodium colistimethate (colistimethate) which is also known as colistin methanesulphonate (8, 9). The terms colistin and colistimethate are not interchangeable. Colistimethate is not stable either *in-vitro* nor *in-vivo*, and is hydrolyzed when in aqueous solution at 37°C, e.g., in plasma or broth, to a series of derivatives plus colistin, which have increased antibacterial activity (9, 10). The rate of hydrolysis depends on physical conditions such as pH and temperature (11). Colistimethate is the inactive pro-drug of colistin (10).

Pharmacokinetics of colistin:
The pharmacokinetics of colistin has been studied using high-pressure liquid chromatography (HPLC) (9, 15). Most studies on the pharmacokinetics have been in patients with cystic fibrosis. Such studies may not be applicable to other patient groups e.g., half-lives in cystic fibrosis patients may be shorter (20). A study involving cystic fibrosis patients showed the half-life of colistimethate to be 2.1hrs, and that of colistin to be 4.2 hrs (11), while another study in this group of patients found a colistin half-life of between 2.3 hours to 6.4 hours (9).

ritically ill patients is available in studies performed on ne of the first studies of this sort in 2005, levels were assessed in a critically ill patient undergoing continuous venovenous haemodialysis (CVVHD) (19). In this study, conversion of colistimethate to colistin was rapid and the half-lives of colistimethate and colistin were 6.8 and 7.5hrs respectively (19). The study by Markou et al found half-lives of colistin in critically ill patients at steady-state with various degrees of renal dysfunction to be between 3.8-9.5 hours (15). Plachouras et al estimated a typical half-life of colistin in critically ill patients to be 14.4 hours (20). This study also found that colistin clearance is not affected by creatinine clearance and thus colistin appears to be cleared by non-renal routes. The protein binding of colistin is reported as being low. A study in rats found that protein binding was about 55% in rat plasma (18). Peak serum levels after intravenous administration occur within 1 hour (15). Higher peak levels tend to occur in renal failure patients (11).

Needless to say, the pharmacokinetic data relating to colistin are diverse, with no consensus on kinetic parameters.

Pharmacodynamics of colistin:
Colistin has rapid bactericidal activity, and is bactericidal in a concentration-dependent manner (6, 9, 12, 17). Studies on the post antibiotic effect (PAE) of colistin have shown some concerning results indicating that even at high MICôs there may be hetero-resistant sub-populations in a number of the strains studied. The strains also showed unfavorable, unexplainable negative PAEôs (17) suggesting that mono-therapy or extended-interval dosage regimens (e.g., 24 hours) may not be optimal (17).

Dosing of colistin:
Due to concerns of drug resistance and therapeutic failure on lower doses there has been a trend to use maximum doses recommended for colistin (Table 1). The dose of colistin is based on international units (IU). One ampoule contains 80mg which is equivalent to 1 million IU. Various formulations of colistin are available in different countries and dose recommendations can be confusing as these are based on mg/kg dosing. International Units are preferable to avoid confusion and attempt to standardize dosing (5). Current recommendations in the UK and the USA recommend doses of up to an equivalent of 2MU per dose, either 12 hourly, 24 hourly or 36 hourly according to renal impairment (5). Maximum doses of colistin stated in the Coly-Mycin® package insert are 3MU at the recommended time intervals.

The microbiological and clinical outcomes of this maximum dose have not yet been evaluated. The concern with this increase in dose is the concomitant increase in side effects particularly nephrotoxicity as reported in a study using a similar high dose regimen (14). Studies by Plachoras et al show no nephrotoxicity even with very high loading doses. It is possible that failure of colistin therapy could be due to inadequate levels in patients with renal failure receiving adjusted doses or the effect of dialysis on the drug levels.

The Serum Bactericidal Test (SBT):
This is an *in-vitro* test that attempts to measure the *in-vivo* activity of the antimicrobial agent. The highest titre (greatest dilution of the patientôs serum) that exerts a bactericidal effect on the

This method of testing incorporates the properties of the antimicrobial agent in question (1, 22). The clinical relevance of SBT testing has been questioned (16).

A study performed at the National Taiwan University hospital undertook to determine the predictive value of the SBT for mortality in patients infected with multi-drug-resistant *A.baumannii* (13). Patients were treated with various antibiotics/antibiotic combinations including carbapenems, ciprofloxacin, aminoglycosides and sulbactam. Colistin was not used in this study. The authors showed that peak serum bactericidal titre negatively correlated with mortality rate (correlation coefficient -0.43). The survival rate in patients with a peak SBT titres of Ó16, Ó8, Ò4 was 100%, 87.5% and 42.4% respectively (p=0.001). Given this strong association, we believe that it will be valid and clinically relevant to compare SBTôs in patients on standard and renally adjusted doses of colistin in order to determine the efficacy of adjusted renal dosage (13).

Materials and Methods

Patients:

Inclusion criteria**:**

Any patient at Groote Schuur Hospital in Cape Town, South Africa requiring colistin therapy either as empiric therapy or for a confirmed infection with a pan drug-resistant organism during the period of June 2009 to March 2010.The decision to commence colistin was made by the attending physician.

Exclusion criteria:

Patients who had lipaemic or haemolysed serum as this could affect the interpretation of SBTôs that were determined according to visual turbidity. Patients or family members who declined consent to participation.

Sample Collection (Clinical samples and patient information):

Colistin was started according to the maximum dosages shown in Table 1. Initial blood samples were taken before colistin was commenced and then 2-3 days after the initial dose was given to allow a steady-state to be achieved (9).

Sample size:

In order to determine a two-fold difference in SBT level, which is considered significant, between the two groups (standard dose and renal dose) with 80% power and type 1 error of 0.05, seventeen patients were required per group.

Blood samples were collected in clot activating tubes. Patients were divided into two groups according to their renal function. Creatinine clearance was estimated by calculation using the Cockcroft-Gault formula (20). This required knowledge of the ideal body weight (IBW) which was calculated using the Broca Index [IBW for men= (height in cm-100), IBW for women= (height in cm -105)].

renal function

	Highest Doses recommended for Coly-Mycin® (26)	**Doses recommended by Sanford Guide to Antimicrobial therapy 2008** (27)
Cr Clearance ml/min	**Dose**	**Dose**
Normal renal function:	3 million U 8 hourly	1-2 million U 8 hourly
50-90	3 million U 12 hourly	2 million U 12 hourly
10-50	3 million U daily	2 million U daily
<10	3 million U 36 hourly	2 million U 36 hourly
Anuric	1.5 million U after each episode of dialysis	1 million U after dialysis
CVVHD	3 million units 12 hourly (12)	2-3 million 48 hourly (1)

Group 1 were patients with normal renal function defined as a clearance of ≥ 90 ml/min. Group 2 included patients with renal impairment not requiring dialysis as well as patients requiring dialysis. Patients were sampled after they reached steady-state. Each colistin dose was administered as a short infusion over 30 minutes. Doses used were the highest doses recommended for Coly-MycinÊ . Dosing of patients in Group 1 was 8 hourly. Each patient had a sample taken before colistin was started (this is the colistin-free control). Blood was drawn at 30min, 60min, 90min (to ensure peak determination) after completion of the colistin infusion, then at 4 hours and 8 hours (to demonstrate the trough level before the next dose was administered).

Group 2: dose frequency varied according to the degree of renal impairment (Table 1). Blood was drawn at 30min,60min and 90 min and then every 4 hours until the next dose was due i.e., at 4 hours, 8 hours and 12hours (trough level) if the dose frequency was 12 hourly for example. The degree of renal impairment, dose adjustment and type of dialysis (if utilized) was documented in all cases. Blood samples were placed on ice immediately (to terminate the conversion of colistimethate to colistin) and taken directly to the laboratory where the serum was first separated from the red blood cells in a refrigerated centrifuge (MISTRAL 3000i) at 3000 rpm (664 x g) for 5min and then aliquoted into respective 1.5ml Eppendorf tubes (MERCK, South Africa) labeled according to time point drawn. The extra blood was used if the test required repeating, thus avoiding thawing and re-freezing the samples. Each sample was checked for sterility, i.e., growth of any organism that may affect turbidity of results. Serum was plated onto blood agar plates and incubated overnight at 35°C to exclude contamination. The serum was stored at -70°C. It has been shown that colistin is stable when frozen (18). Samples were thawed to room temperature just before processing.

Laboratory setting:
Microbiology Laboratory, National Health Laboratory Service (SANAS: South African National Accreditation Service) accredited,
Groote Schuur Hospital, Cape Town, South Africa

prevent premature thawing at room temperature. Testing was performed in sterile, plastic, flat-bottomed MICROLON ® microplate 96 well trays (Lasec SA, South Africa). The final volume in each well was 0.1ml.

Inoculum preparation and inoculation of wells:
The *ATCC BAA-1605* strain available did not meet the study criteria thus a clinical strain isolated from a patient, that was resistant to all antibiotics tested except colistin and tobramycin was used. In no clinical situation were these two antibiotics used together.

The degree of resistance was characterized (by doing Minimum Inhibitory Concentration testing using an Epsilometer E-TEST). This standard strain was used to ensure that results can be compared to each other with no bias due to variation in strain MIC.

The CLSI (Clinical and Laboratory Standards Institute) recommendations required that the final inoculum of the reference strain in each test well were 5×10^5 cfu/ml. In order to achieve this concentration, preparation of the reference strain was required, as follows:

First, the reference strain of *Acinetobacter baumannii* was subcultured three times on standard blood agar plates to ensure that the organism had optimal growth and metabolic status prior to drug exposure (16).
Next, suspensions of the organism with a turbidity equivalent to that of a 1 McFarland standard was prepared after suspending the colonies into a pre-warmed (35°C) Tripticase Soya Broth (TSB -Greenpoint NHLS Media Laboratory, South Africa). These were incubated for 6 hours to ensure log-phase growth.
Finally, the reference strain was diluted to achieve the optimal concentration. As a 1 Mcfarland standard contains approximately 3×10^8 cfu/ml, and the final concentration of the reference strain in each well once inoculated was 5×10^5 cfu/ml, a 1:30 dilution of the 1 McFarland standard was performed by adding 0.1ml of the reference strain suspension to 2.9ml of broth. This yielded an inoculum density of 1×10^7 cfu/ml, which was further diluted 1:20 by adding 5ul of this organism suspension to the 100ul (serum/TSB mixture) in the test well. This yielded a final inoculum of 5×10^5 cfu/ml in each well.

For each timed sample from a patient e.g. patient A; Initial sample, a single row of a microwell plate (MWP) was used, with one MWP per patient. Firstly, 100uL of TSB was dispensed into wells 1-2 and 4-10 in a single row. Next, 5uL of ATCC strain was dispensed into the 1st TSB-containing well, to serve as the positive growth control. The second well served as the negative growth control. Into the 3rd and 4th wells, 100uL of neat serum was dispensed. Serial doubling dilutions began from the 4th well using standard techniques with the final well for every sample being a 1:128 dilution. Next 5uL of the ATCC strain was added to wells 3 to 10, achieving a final volume of 105uL in all 10 wells.
According to the CLSI document the volume of organism suspension added to the well must not exceed 10% of the total volume of the well as this may affect the dilution (16).

For the next row, the above method was repeated for the next timed sample collected from the same patient e.g. patient A; 30 min draw. Once all the rows were inoculated for a patient

ollected per patient), the microwell plate was incubated in

Reading of results:
Wells were only read if there was definite turbidity in the positive growth control and no growth in the negative growth control. Twenty microlitres of fluid from every well containing no growth was sub-cultured onto Mueller-Hinton agar to assess the bactericidal activity of the drug. The plates were incubated in air at 35°C for 18-24 hours. The dilution that demonstrated 99.9% killing, i.e., 20ul of the 5 X 10^5 cfu/ml dilution containing fewer than 10 colonies on the plate, was the bactericidal titre.

Results:
Thirty four patients were enrolled in this study, seventeen patients with normal renal function (Group 1) and seventeen with renal impairment (Group 2). Of the seventeen with renal failure six were anuric and required dialysis. Types of dialysis included Intermittent Haemodialysis (IHD) and Sustained Low Efficiency Dialysis (SLED). There were 3 patients in each dialysis sub-group. Demographic and clinical data for the two groups are shown in Table 2.

Table 2: Patient Demographic and Clinical details

Patient N=34	Cr Cl	Ward	Recommended dose	Dose administered	Age	M/F	Current/recent concurrent antibiotics	Actual weight (kg)	Ideal body weight (kg)	Day of sampling	Reason for admission
Patients with normal renal function: Group 1											
Patient 1	116	D12 ICU	3MU 8hrly	3MU 8hrly	28	M	CAZ,GEN		70	2	Perforated appendix
Patient 2	119	C27 ICU	3MU 8hrly	3MU 8hrly	42	F	SXT,ETP	60	60	6	Severe pneumonia
Patient 3	197	C27 ICU	3MU 8hrly	3MU 8hrly	56	F	ETP	60	60	4	Pontine lesion
Patient 4	149	D12 ICU	3MU 8hrly	3MU 8hrly	35	M	none	55	60	4	Severe pneumonia
Patient 5	210	E26	3MU 8hrly	3MU 8hrly	35	F	SXT		70	3	Pancreatitis
Patient 6	105	D12 ICU	3MU 8hrly	3MU 8hrly	53	F	AMC	80	65	5	Pancreatitis
Patient 7	249	C27 ICU	3MU 8hrly	3MU 8hrly	31	F	IMI		70	4	Bacterial meningitis
Patient 8	250	G12 D12	3MU 8hrly	3MU 8hrly	61	F	none		70	3	Pneumonia
Patient 9	201	D12 ICU	3MU 8hrly	3MU 8hrly	25	M	AMC		70	3	Polytrauma
Patient 10	205	F26	3MU 8hrly	3MU 8hrly	27	F	none		65	6	Renal stone
Patient 11	150	D13 ICU	3MU 8hrly	3MU 8hrly	47	M	none		70	4	Subdural haemorrhage
Patient 12	155	C27 ICU	3MU 8hrly	3MU 8hrly	46	F	TZP,CIP		70	3	Acute spinal cord injury
Patient 13	143	D12 ICU	3MU 8hrly	3MU 8hrly	51	M	IMI	75	70	3	viral meningitis
Patient 14	97	C27 ICU	3MU 8hrly	3MU 8hrly	38	M	none		70	6	Sigmoid tumor
Patient	298	C27S	3MU 8hrly	3MU 8hrly	27	M	VAN, AZM		65	5	Spinal

											trauma
				3MU 8hrly	27	F	none	70	65	3	Gastric carcinoma
Patient 17	334	D12 ICU	3MU 8hrly	3MU 8hrly	24	M	IMI	65	75	3	Abdominal trauma

Patients with impaired renal function: Group 2											
Patient 18	69	D12 ICU	3MU 12hrly	3MU 8hrly	68	F	ETP		70	5	Motor vehicle accident
Patient 19	AN	C27 ICU	1.5MU A/D	1.5MU after dialysis	32	F	SXT	46	65	3	Liver failure
Patient 20	70	C27 ICU	3MU 12hrly	3MU 12hrly	59	M	AMK	75	70	3	Perforated appendix
Patient 21	43	G5	3MU 24hrly	3MU 12hrly	41	M	ETP		70	4	Tuberculosis
Patient 22	AN	C27 ICU	1.5MU A/D	1.5MU after dialysis	65	M	ETP	71	70	4	Diabetes with sepsis
Patient 23	84	D12 ICU	3MU 12hrly	3MU 8hrly	57	M	none		70	4	Motor vehicle accident
Patient 24	74	D12 ICU	3MU 12hrly	3MU 24hrly	48	F	IMI	59	60	6	Infective endocarditis
Patient 25	AN	D12 ICU	1.5MU A/D	1.5MU after dialysis	40	F	CAZ		60	3	Renal failure
Patient 26	46	D22 ICU	3MU 24hrly	3MU 8hrly	56	F	TZP		70	5	Abdominal mass
Patient 27	AN	D12 ICU	1.5MU A/D	1.5MU after dialysis	31	F	none	80	65	5	Septic abortion
Patient 28	66	F17	3MU 12hrly	3MU 8hrly	57	M	AMK	64	75	5	Stomach carcinoma
Patient 29	18	F4	3MU 24hrly	3MU 8hrly	61	M	IMI, VAN		65	3	Abdominal sepsis
Patient 30	83	C12	3MU 12hrly	3MU 12hrly	36	M	none		70	4	Polytrauma
Patient 31	AN	E26	1.5MU A/D	1.5MU after dialysis	22	M	IMI, VAN		70	3	Pneumonia
Patient 32	67	F16	3MU 12hrly	3MU 8hrly	63	M	VANC		70	5	Septic wound
Patient 33	29	D12 ICU	3MU 24hrly	3MU 36hrly	41	F	IMI	67	55	6	Nephrotic syndrome
Patient 34	AN	D12 ICU	3MU 12hrly and 1.5MU A/D	3MU 12hrly	59	M	CIP, MTZ		70	7	Pancreatitis

CAZ, ceftazidime; GEN, gentamicin; SXT, trimethoprim-sulphamethoxazole; AMC, amoxicillin-clavulanate; IMI, imipenem; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; VAN, vancomycin; AZM, azithromycin; AMK, amikacin; MTZ, metronidazole AN: Anuric

Table 3 shows a summary of the SBT results. Tables 4 and 5 show the SBTôs obtained at peak and troughs respectively, in the two groups of patients. Sixteen of the seventeen patients (94.1%) in both Group 1 and 2 had a SBT of Ó16 at or near the peak (30, 60 or 90min). Eleven of the seventeen patients (64.7%) in Group 1, compared to 5/17 (29.4%) in Group 2 had SBTôs Ó16 for the entire duration of time until the next dose was administered (p= 0.086). Of the Group 2 patients 2/17 did not have all of the samples taken but the SBTôs at the other sampling times were all Ó16. There were six anuric patients on dialysis in Group 2. All achieved peak SBTôs of Ó16 at peak, however, no anuric patient on dialysis achieved a SBT Ó16 for the duration of drug exposure. Finally, 14/17 (82.4%) in Group 1 had SBT Ó16 at trough (just before next dose), compared to 6/17 (35.3%) in Group 2 which is statistically significant (p= 0.015). None of the six anuric patients on dialysis achieved a trough SBT of Ó16.

e features and Serum Bactericidal Titres in Group 1

	Group 1: Normal renal function (N=17)	Group 2: Renal impairment (N=17)	
M:F	1: 1.1	1: 1.4	p= 0.731
Age (yr): Mean	38 (24-61)	49 (55-75)	p= 0.021
Ideal Body weight (kg)	67.3 (65-75)	67.3 (55-75)	p= 1.0
Creatine Clearance: (ml/min)*	182.4 (97-354)	38.2 (0-84)	p= <0.0001
No. of patients in septic shock during sampling (%)	1/17 (6)	2/17 (11.8)	p= 0.581
Day of sampling: Mean	3.9 (2-6)	4.4 (3-7)	p= 0.276
No. of patients on other concurrent or recent antibiotics (%)	11/17 (64.5)	14/17 (82.4)	p= 0.437
No. of patients who had SBT ≥ 16 at peak (30-90min) (%)	16/17 (94.1)	16/17 (94.1) 6/6 (100) Anuric sub-group	p= 1.0
No. of patients who had SBT ≥16 for the entire duration of sampling (%)	11/17 (64.7)	5/17 (29.4) but 2 patients NK 0/6 (0) Anuric sub-group	p= 0.086
No. of patients with trough SBT of ≥16 (%)	14/17 (82.4)	6/17 (35.3) but 3 ND 0/6 (0) Anuric sub-group but 1 ND	p= 0.015

NK: (Not known) Patients who had a SBT of 16 or above for all other samples tested for that patient, but at certain sampling times had no blood taken (ND)
ND: (Not done) Sample not done due to either patient refusal or inability to draw blood or patient demise
*** calculated using Cockroft-Gault formula which uses ideal body weight (IBW)**

…res (SBT)

Group 1: Normal renal function		Group 2: Renal impairment (D – haemodialysis)	
Patient	**Titre**	**Patient**	**Titre**
Patient 1	16	Patient 18	Ó128
Patient 2 *	64	Patient 19 *	16
Patient 3 *	64	Patient 20 *	32
Patient 4	64	Patient 21 *	Ó128
Patient 5 *	64	Patient 22 *(D)	32
Patient 6 *	16	Patient 23	8
Patient 7 *	16	Patient 24 *	64
Patient 8	32	Patient 25 (D)	16
Patient 9	16	Patient 26	128
Patient 10	Ó128	Patient 27 *(D)	128
Patient 11	4	Patient 28 *	128
Patient 12	32	Patient 29 *	128
Patient 13 *	16	Patient 30	16
Patient 14	16	Patient 31 (D)	64
Patient 15	32	Patient 32 *	128
Patient 16	16	Patient 33	64
Patient 17	16	Patient 34 *	16

(D): Patient on Dialysis
***: Patient Died**

Table 5: Trough Serum Bactericidal Titres (SBT)

Group 1: Normal renal function		Group 2: Renal impairment (D – haemodialysis)	
Patient	**Titre**	**Patient**	**Titre**
Patient 1	8	Patient 18	128
Patient 2 *	64	Patient 19 *	8
Patient 3 *	32	Patient 20 *	8
Patient 4	32	Patient 21 *	128
Patient 5 *	16	Patient 22 *(D)	8
Patient 6 *	16	Patient 23	16
Patient 7 *	16	Patient 24 *	ND
Patient 8	32	Patient 25 (D)	2
Patient 9	16	Patient 26	64
Patient 10	32	Patient 27 *(D)	2
Patient 11	128	Patient 28 *	32
Patient 12	16	Patient 29 *	64
Patient 13 *	8	Patient 30	ND
Patient 14	8	Patient 31 (D)	ND
Patient 15	16	Patient 32 *	8
Patient 16	16	Patient 33	4
Patient 17	16	Patient 34 *	8

(D): Patient on Dialysis
***: Patient Died**

num recommended dose and all anuric patients on dialysis.

Only 8 of the 17 patients in Group 2 received the maximum recommended dose (Table 6). Of the patients who received this dose, 16/17 (94.1%) in Group 1 and all of patients in Group 2 achieved an SBT at peak of Ó16 (p= 0.48). Twelve of the seventeen (70.6%) in Group 1 had an SBT Ó16 for the duration of dosing, compared to none in Group 2, which was significant (p=0.001). Fourteen of the patients in Group 1 (82.4%) compared to none in Group 2 had Troughs of Ó16 (p=0.001).

Of the patients in Group 2, seven of the seventeen patients received a higher dose than maximum recommended dose. Four of these seven patients had doses recommended 12 hourly but were given their dose 8 hourly. Two of the seven patients who were supposed to get a dose every 24 hours received their medication 12 hourly. One of the seven patients who required 24 hour dosing received their colistin 8 hourly. Of these patients 6/7 (85.7%) reached a SBT of Ó16, 5/7 (71.4%) achieved this for the entire duration, and 6/7 (85.7%) at Trough.

Only two patients in Group 2 were dosed less frequently than recommended. One patient was supposed to get a dose 12 hourly but got it 24 hourly and the other was to get it every 24 hours but received it every 36 hours. In the less frequently dosed group 2/2 had SBT Ó16 at peak but 0/2 had SBT Ó16 for the full dosing duration and trough.

Mean day of sampling after commencement of dose was 3.9 (2-6) in Group 1, compared to 4.4 (3-7) days in Group 2 (p= 0.276). The mean age in Groups 1 and 2 respectively were 38 (24-61) and 49 (55-75), (p= 0.021). Male to female ratios were 1:1,1 and 1: 1,4 in Group 1 and Group 2 respectively (p= 0.713). The mean ideal body weight for the two groups was equal (p= 1.0). The number of patients in septic shock in Group 1 and 2 was 1/17 and 2/17 respectively (p= 0.581). The number of patients on concurrent antibiotics was 11/17 and 14/17 in Group 1 and Group 2 respectively (p= 0.437). The number of patients on concurrent imipenem in the two groups was similar, 3/17 in Group 1 and 4/17 in Group 2 (p= 0.672).

Table 6: Comparison of SBT's in Group 1 and Group 2 according to dose administered

	Group 1: Normal renal function	**Group 2: Renal impairment**
No. (%) receiving maximum recommended dose:	**17/17 (100)**	**8/17 (47.1)** p=0 .0005
Peak SBT ≥ 16:	16/17 (94.1)	8/8 (100) p=0.48
Entire duration SBT ≥ 16:	12/17 (70.6)	0/8 but 1 NK p=0.001
Trough SBT ≥ 16:	14/17 (82.4)	0/8 but 2 ND p=0.001
No. (%) receiving lower than recommended dose:	**0/17 (0)**	**2/17 (11.8)**
Peak SBT ≥ 16:		2/2 (100)
Entire duration SBT ≥ 16:		0/2 (0) but 1 NK
Trough SBT ≥ 16:		0/2 (0) but 1 ND

		7/17 (41.2)
Peak SBT ≥ 16:		6/7 (85.7)
Entire duration SBT ≥ 16:		5/7 (71.4)
Trough SBT ≥ 16:		6/7 (85.7)

NK: (Not known) Patients who had a SBT of 16 or above for all other samples tested for that patient, but at certain sampling times had no blood taken (ND)
ND: (Not done) Sample not done due to either patient refusal, inability to draw blood or patient demise
*** calculated using Cockroft-Gault formula which uses ideal body weight (IBW)**

SBTôs were performed on each patientôs serum that was taken before the commencement of colistin . In both Groups, 2/17 had SBTôs of more than 1. Titres of 2 and 4 in Group 1, and 2 and 8 in Group 2.

Overall mortality in Group 1 was 6/17 (35%) and in Group 2 was 10/17 (59%). Of the patients on dialysis 50% (3/6) died. We were not able to establish whether death was directly attributable to sepsis or not as mortality in many cases was not temporarily related to the sepsis. Statistical analysis and comparison of death and SBTôs achieved was thus not done.

Discussion:

Many factors play a role in determining the clinical outcome of a host invaded by a pathogen. These include: host factors, drug factors and organism factors (16). In this study we used a standard organism in order to assess the drug/host interaction specifically. While host variables could not be eliminated, we were able to show that they were not significantly different in the two groups studied. It can be assumed therefore that the differences seen in SBTôs are largely due to differences in drug administration.

All patient variables, except age were shown not to be significantly different in the two groups. One of the host factors that could not be controlled, the bactericidal effect of human serum alone required assessment. This phenomenon occurs due to complement mediated lysis (3). Insignificant SBTôs were found with human serum alone without colistin. Other factors that could not be predicted or standardized related to *in-vivo* conditions, e.g., pH that may affect conversion of colistimethate to colistin as well as improvement/deterioration of renal function during the sampling process which may alter drug clearance.

The collection, storage and processing of samples was standardized. Samples were frozen immediately after centrifugation in a refrigerated centrifuge to minimize hydrolysis of the drug. All laboratory procedures were carried out in a temperature controlled environment, with only one unfrozen specimen at a time.

Sampling patients at exactly the same time after they had achieved steady-state was difficult to standardize for logistic reasons such as availability of phlebotomy staff and patients in theatre at

o statistically significant difference in the mean day of roups.

The strain of *Acinetobacter* used in the study had a colistin MIC of 0.25ug/ml. For an *Acinetobacter* isolate to be regarded as sensitive it needs to have an MIC of Ò2ug/ml. Possible clinical *Acinetobacter* isolates may have variable colistin MICôs, and by choosing a very sensitive strain such as this, the SBT obtained would theoretically represent the best case scenario.

A possible confounder in this study was the fact that many patients were on various concurrent antibiotics. Synergy studies show that there is no marked synergy of a polymyxin in combination with imipenem, rifampicin or azithromycin against certain strains of carbapenemase producing *A.baumannii* isolates (25).
A few studies have shown synergy when certain *A.baumannii* isolates are treated with colistin and rifampicin (23, 24). None of the patients in this study were on rifampicin and colistin concurrently, however, 3/17 patients in Group 1 were on concurrent imipenem and 4/17 patients in Group 2 were on concurrent imipenem (p=0.672). All patients on imipenem had peak SBTôs of Ó16. Timuryanak et al and other authors demonstrated that synergy occurs with colistin and meropenem/azithromycin, as well as partial synergy when colistin was combined with doxycyline against multidrug resistant organisms. No patients in this study were on meropenem/doxycycline or azithromycin (24).

Some patients had recently received other broad-spectrum antibiotics and others had renal failure which can affect clearance of these drugs. To minimize the effect of possible synergy/antagonism, a characterized strain of *Acinetobacter baumannii* that was resistant to all locally available antimicrobials was used. This strain was sensitive to colistin ensuring that there would be a minimal effect on the SBT from the other concomitant antibiotics. The number of patients on concurrent antibiotics in the two groups was not statistically different.

Serum Bactericidal Titres of patients receiving doses of colistin recommended for normal renal function were compared to those receiving adjusted doses due to renal impairment. Based on previous data showing an association between mortality and the SBT, the greatest benefit when using an antibiotic was found when the SBT at peak was 16 or more (13). No information exists on what the significance of a SBT is at other time points of the dosing interval. Although a SBT predicts bacterial eradication, the clinical relevance of a SBT remains controversial (16). In vitro bacterial eradication cannot predict clinical cure. One must remember that biological assays such as this are unable to differentiate the amount of colistin present in the sample at the time of collection from the colistin formed in vitro by hydrolysis of colistimethate during the microbiological assay (2). Bias was minimized by treating all samples in the same manner and using the SBT to compare the two groups. Microbiological assays are unable to directly determine the serum concentration of the active components of colistin achieved with a particular dose. It is currently not known which metabolites of colistimethate are active thus when using a microbiological assay one is able to determine the collective effect of all the active metabolites in the sample.

he maximum recommended dose may be sufficient in k. Group 2 includes all of the anuric patients who also obtained SBT peaks of Ó16, all of whom received the recommended maximum dose. The major difference was noted when comparing the SBT at trough and those achieved for the entire duration of dosing. These results suggest that the maximum doses recommended may not be sufficient to obtain high SBTôs at times other than the peak. This has not yet been proven to be associated with clinical failure, but may explain the poor response observed in some patients receiving colistin. Only the SBT of Ó16 at peak has been shown to be a marker of improved outcome. The results of the SBT at peak in both groups including the dialysis patients were shown to be favourable. The concern is the possible lower levels achieved, as suggested by the lower SBTôs found for the duration of drug exposure. These low SBTôs were also found at trough in Group 2, especially in the dialysis group. These findings may explain poorer outcomes observed in some patients on dialysis and may suggest that colistin is removed by dialysis and that higher doses may need to be considered in these patients. Whether higher SBT levels at trough and throughout the dosing period are important to patient response to the colistin remains to be seen.

Not all patients in Group 2 received the recommended maximum dose and thus could not be included in the analysis of this dosing strategy, but it was interesting to see that all dosing strategies, maximum recommended, higher and lower than recommended, achieved acceptable SBTôs at peak. All groups had low SBTôs at trough and for the entire dosing duration except most of the patients in Group 1 and those patients in Group 2 who were dosed higher than the maximum recommended dose. One may argue that this indicates that higher doses are necessary in patients with renal impairment if trough levels and levels achieved throughout dosing are found to be important. Of these patients, 7/17 patients were given higher doses than recommended and 2/17 were given lower doses. The miscalculations could have resulted due to calculation of creatinine clearance using the actual body weight (ABW) rather than ideal body weight (IBW). IBW and not ABW should be used to calculate creatinine clearance. Using the ABW in obese patients may lead to overdosing and nephrotoxicity according to a study by DeRyke in AAC July 2010 (21).

The mortality data has been shown for completeness however it must be stressed that the SBTôs achieved were determined using a characterized laboratory strain of *Acinetobacter baumannii*. The patientôs own isolate was not used and not all patients cultured an *Acinetobacter*. Some patients cultured *Pseudomonas* and others were started on empiric therapy. Due to many other complicating patient factors and variable times of death relating to the sepsis requiring colistin therapy, mortality could not be directly attributed to underdosing with colistin.

The prime reason for having undertaken this study is the lack of knowledge of the kinetics of colistin and its active metabolites. This has led to uncertainty about the efficacy of doses given to patients with renal impairment and those on dialysis. The use of a pharmacological assay on only one of the many possible active components of colistin may be misleading. The SBT, in contrast, is able to determine the actual activity of all the active metabolites that are having a bactericidal effect and thus provides at least *in-vitro* evidence of bactericidal activity. Whether this can then be correlated with clinical outcome is a separate issue. Ideally an SBT at which the drug is known to be effective, that correlates with clinical outcome (in the same way as a peak

es) would be useful. This could then be used as a
rrelate of efficacy. Regrettably, none of this data is
available for colistin.

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Acknowledgements

This work was supported by the National Health Laboratory Service Research Trust, I would like to thank Sister Reitz and Gillian Peat for assisting with sample collection and Dr Ivan Joubert for the study design, clinical input as well as enthusiasm, Ingrid Wilson for obtaining outcome data, Reene Naidoo and Marvin Hsiao for assistance with statistics and Dr Sheetal Manicklal, Prof Mark Nicol and Dr Stephen Oliver for proof reading and recommendations.

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Supporting Documentation

Documents submitted:

- Consent form
- Additional Table: *Table 7* not included in the Manuscript
- Ethics approval letter from the Faculty Research ethics committee

Appendix:

- Journal of Clinical Microbiology: Instructions to Authors

GROOTE SCHUUR AND RED CROSS CHILDREN'S HOSPITALS
Department of Clinical Laboratory Sciences
Groote Schuur Hospital
OBSERVATORY
SOUTH AFRICA

NATIONAL HEALTH LABORATORY SERVICE

NATIONAL HEALTH LABORATORY SERVICE

INFORMED CONSENT FORM FOR: [redacted]

Consent is for patients in the Respiratory/Surgical Intensive Care Units at Groote Schuur Hospital, Cape Town. Patients will be on colistin treatment for suspected/confirmed infection with *Acinetobacter baumannii*. Patients in the study may have various degrees of kidney dysfunction.

PRINCIPAL INVESTIGATOR: Dr Justyna Wojno
NATIONAL HEALTH LABORATORY SERVICE, GROOTE SCHUUR HOSPITAL
PROPOSAL TITLE: Serum bactericidal activity of colistin against *Acinetobacter baumannii* in patients with normal versus impaired renal function

INFORMATION SECTION:
There is a bacterium called Acinetobacter that can sometimes cause infections in people who are in an ICU (Intensive care unit). These bacteria have become very resistant to many of the antibiotics that we normally use in the hospital. This means that, if you do get an infection with this organism, we would need to use an antibiotic called colistin. This is an old antibiotic but it hasn't been used much until recently and, although there are recommended doses, we need to find out more about the best dose to use in patients whose kidneys are not working properly. For this reason we would like to collect blood from patients who are getting colistin so that we can conduct tests that would help us to check that we are giving the best dose.
With this study the investigators will hopefully have more knowledge of colistin and thus be able to treat patients more effectively in the future.

PROCEDURE: Blood samples will be taken from patients, using a sterile procedure, at intervals throughout the day. This will be for a period of 12-24 hours only. The total number of samples taken will be about 6.

PATIENT CONFIDENTIALITY: Under no circumstance will the identity of the patient be disclosed to anyone. All samples that are collected with have a number, and the patient's name will be omitted.

RISKS AND DISCOMFORTS: The risk to a patient when a doctor/nurse draws blood is minimal. The patient may experience some discomfort during the procedure. To avoid repeated discomfort, a temporary Safelock device may be placed in the vein so that blood can be drawn through this during the duration of the sampling.

RIGHT TO WITHDRAW FROM THE STUDY: The patient may at any point of the study decide to withdraw. This will not influence the patient's further management.

I HAVE READ THE ABOVE INFORMATION/OR IT HAS BEEN READ TO ME. MY QUESTIONS HAVE BEEN ANSWERED. I GIVE MY CONSENT FOR THE PROCEDURE AND UNDERSTAND THAT I CAN AT ANY STAGE DECIDE TO WITHDRAW MYSELF/FAMILY MEMBER FROM THE STUDY

signature removed

Name of Participant:
Name of Family member/Guardian: **DATE:** 2/6/09

SIGNATURE Of PARTICIPANT/FAMILY MEMBER/GUARDIAN:

all time points Group 1 and Group 2

	No ab	Before dialysis	30 min	60	90	240	480	720	1440	2160
Group 1: Normal renal function										
Patient 1	1		16	16	16	16	8			
Patient 2	4		32	32	64	64	64			
Patient 3	1		32	32	64	64	32			
Patient 4	2		16	64	64	32	32			
Patient 5	1		32	64	64	32	16			
Patient 6	1		16	16	16	16	16			
Patient 7	1		16	16	16	16	16			
Patient 8	1		32	32	32	32	32			
Patient 9	1		16	16	16	16	16			
Patient 10	1		64	64	128	64	32			
Patient 11	1		4	4	2	32	128			
Patient 12	1		16	32	32	16	16			
Patient 13	1		4	8	16	8	8			
Patient 14	1		16	8	16	8	8			
Patient 15	1		16	16	32	16	16			
Patient 16	1		8	16	16	16	16			
Patient 17	1		16	16	8	16	16			

				60	90	240	480	720	1440	2160
Group 2: Renal impairment										
Patient 18	2		128	128	128	128	128			
Patient 19	1	8	16	16	16	16	8	ND		
Patient 20	1		16	32	16	16	16	8		
Patient 21	1		128	128	128	128	128	128		
Patient 22	1	8	8	16	32	16	16	32		
Patient 23	1		8	8	4	32	16			
Patient 24	1		64	64	64	64	64	32	ND	
Patient 25	1	2	4	8	16	16	16	8		
Patient 26	1		128	64	128	128	64			
Patient 27	1	2	16	64	128	64	ND	32		
Patient 28	8		32	128	32	32	32			
Patient 29	1		64	128	128	64	64			
Patient 30	1		8	8	16	32	8	ND		
Patient 31	1	ND	32	32	64	64	64	ND	ND	ND
Patient 32	1		16	128	128	32	8			
Patient 33	1		32	64	64	32	32	ND	16	4
Patient 34	1	8	16	16	16	16	8	ND		

UNIVERSITY OF CAPE TOWN

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Research Ethics Committee
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23 March 2009

REC REF: 135/2009

Dr JM Wojno
Microbiology
NHLS

Dear Dr Wojno

PROJECT TITLE: SERUM BACTERICIDAL ACTIVITY OF COLISTIN AGAINST ACINETOBACTER BAUMANNII IN PATIENTS WITH NORMAL VERSUS IMPAIRED RENAL FUNCTION.

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 30th March 2010.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

signature removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

lemjedi