TREATMENT OUTCOMES OF PATIENTS WITH ACINETOBACTER INFECTION; COMPARISON BETWEEN POLYMYXIN VERSUS NON POLYMYXIN BASED THERAPY

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In the name of Allah, the Beneficent, the Merciful.

By the grace of Almighty Allah, I have started and completed this study.

I dedicate this thesis to my father Mr Afzal Jeeawoody, my mother Mrs Bibi Razgia Jeeawoody, my brother Mr Adil Jeeawoody and my sister-in-law Mrs Bibi Sajeda Budaly Jeeawoody who have always been there for me.

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Allah bless you all.

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Introduction: The growing resistance of *Acinetobacter* to almost all commercially available antibiotics is of major concern. Limited therapeutic options are currently available.

Objectives: The aim of the study was to compare the efficacy of sulbactam regime to that of polymyxin B in the treatment *Acinetobacter* infection.

Patients and Methods: This was a retrospective study of case records over one year period (1st January 2018 to 31st December 2018) at the Hospital Universiti Sains Malaysia. Patients of least 18 years old, with clinical and microbiological evidence of *Acinetobacter* infection, were enrolled in the study.

Results: 34 patients received polymyxin and 38 received either ampicillin-sulbactam or cefoperazone-sulbactam. 24 (63.2%) from the nonpolymyxin group achieved clinical success while 13 (38.2%) achieved clinical success in the polymyxin group. 26 patients (68.4%) treated with nonpolymyxin achieved microbiological success compared to 18 (52.9%) treated with polymyxin. Mortality was lower in the nonpolymyxin group with 17 deaths (44.7%) compared to 23 deaths (67.6%) in the polymyxin group. Multiple logistic regression showed that microbiological failure was significantly associated with 30 days in patient mortality.

Conclusion: The most important finding of our study is that sulbactam appears to have a better efficacy compared to polymyxin in treating *Acinetobacter* infection.

Dr Alwi Muhd Besari @ Hashim: Supervisor

Associate Professor Dr Zakuan Zainy Deris: Co-Supervisor

Associate Professor Dr Siti Suraiya Md Noor: Co-Supervisor

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LIST OF ABBREVIATIONS

CFU : Colony forming unit

CRAB : Carbapenem resistant *Acinetobacter baumannii*

CRP : C reactive protein

CSF : Cerebrospinal fluid

ESRF : End stage renal failure

HAP : Hospital acquired pneumonia

HDU : High dependency unit

Hep B : Hepatitis B

Hep C : Hepatitis C

HUSM : Hospital Universiti Sains Malaysia

ICU : Intensive care unit

MDR : Multi drug resistant

MDRAI : Multi drug resistant *Acinetobacter* infection

NEWS2 : National Early Warning Score 2

UKJEH : Unit Kawalan Jangkitan & Epidemiologi Hospital

USM : Universiti Sains Malaysia

VAP : Ventilator associated pneumonia

LIST OF SYMBOLS

%	percent
=	equal to
>	more than
<	less than
≤	less than or equal to
≥	more than or equal to
℃	degrees Celsius
ml	millilitre
vs.	versus
SpO2	saturation in oxygen
CFU/ml	Colony forming unit per millilitre
cells/mm^3	cells per millimetre cube

ABSTRAK

Latarbelakang: Peningkatan ketahanan *Acinetobacter* terhadap hampir kesemua antibiotik yang berada di pasaran merupakan suatu kebimbangan utama. Pada masa ini, terdapat pilihan pengubatan yang terhad.

Objektif: Tujuan utama kajian ini adalah untuk membandingkan keberkesanan amalan sulbactam terhadap polymyxin B dalam rawatan jangkitan *Acinetobacter*.

Kaedah: Ini merupakan kajian retrospektif rekod kes dalam jangkamasa setahun (1 Januari 2018 hingga 31 Disember 2018) di Hospital Universiti Sains Malaysia. Kajian ini melibatkan pesakit yang berumur sekurang-kurangnya 18 tahun, dan mempunyai bukti klinikal dan mikrobiologikal jangkitan *Acinetobacter*.

Keputusan: 34 pesakit menerima polimiksin dan 38 telah menerima sama ada ampicillin-sulbactam atau cefoperazone-sulbactam. 24 (63.2%) daripada kumpulan bukan polymyxin mencapai kejayaan klinikal manakala 12 (38.2%) mencapai kejayaan klinikal dalam kumpulan polymyxin. 26 pesakit (68.4%) yang dirawat dengan bukan polymyxin mencapai kejayaan mikrobiologikal berbanding dengan 18 (52.9%) yang dirawat dengan polymyxin. Kematian adalah rendah dalam kumpulan bukan polymyxin dengan jumlah 17 sahaja (44.7%) berbanding dengan 23 kematian (67.6%) dalam kumpulan polymyxin. Regresi logistik pelbagai menunjukkan bahawa kegagalan mikrobiologikal terkait secara signifikan dengan 30 hari kematian pesakit.

Kesimpulan: Penemuan terpenting kajian kami adalah sulbactam yang sebenarnya lebih berkesan daripada polymyxin dalam merawat jangkitan *Acinetobacter*.

Kata kunci: *Acinetobacter*, polymyxin, sulbactam, berkesan, kematian

ABSTRACT

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Conclusion: The most important finding of our study is that sulbactam appears to have a better efficacy compared to polymyxin in treating *Acinetobacter* infection.

Keywords: *Acinetobacter*, polymyxin, sulbactam, efficacy, mortality

CHAPTER 1

INTRODUCTION

Acinetobacter is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria. It comprises of more than 50 species, most of which are nonpathogenic environmental organisms. The most common infection-causing species is *Acinetobacter baumannii,* followed by *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Acinetobacter baumannii* has the potential of spreading among hospitalized patients by virtue of its ability for exogenous colonization of human body (throat, gastrointestinal tract, skin) and its high tolerance of difficult conditions (survivability in the environment up to 1 month) (Wendt et al. 1997).

The ability of *Acinetobacter* to accumulate diverse mechanisms of resistance, has led to the emergence of strains that are resistant to all commercially available antibiotics (Lolans et al., 2006). *Acinetobacter baumannii* forms part of the ESCAPE organisms, which are predominantly health care-associated organisms that have the potential for substantial antimicrobial resistance (De Rosa et al. 2015, Rice et al. 2008).

In the year 2011, the European and United States Centres for Disease Control and Prevention (ECDC and CDC) joined to propose specific definitions for characterizing drug resistance in organisms that cause many health care-associated infections (Magiorakos et al. 2012). The following definitions were established based on the extent of resistance to antibiotics that would otherwise serve as treatments for *Acinetobacter* (cephalosporins, fluoroquinolones and carbapenems)

- Multidrug-resistant: isolate is non-susceptible to at least one agent in three or more antibiotic classes
- Extensively drug-resistant: isolate is non-susceptible to at least one agent in all but two or fewer antibiotic classes

- Pandrug-resistant: isolate is non-susceptible to all agents

As from the 1980s, the resistant strains of *Acinetobacter* became more and more common causes of nosocomial infections globally (Gaynes et al. 2005, Rhomberg et al. 2007, Tatman-Otkun et al. 2004). Based on a 2009 report of surveillance data from more than 100 centers worldwide (Meropenem Yearly Susceptibility Test Information Collection; MYSTIC), 61 percent of *Acinetobacter* isolates were resistant to ceftazidime and 67 percent were resistant to ciprofloxacin (Rhomberg et al. 2009). Emergent carbapenem-resistant strains have been demonstrated by other worldwide studies with high rates of carbapenem resistance in some locations (Giske et al. 2008, Jean et al. 2011, Manikal et al. 2003, Peleg et al. 2006, Playford et al. 2007). For instance, the prevalence of carbapenem-resistant *Acinetobacter baumannii* at two teaching hospitals in the UK increased from 47 to 77 percent from 2010 to 2012 (Freeman et al. 2015) while in one referral hospital in northern Vietnam, more than 90 percent of isolates were carbapenem resistant (Van et al. 2014). The reported prevalence of carbapenem resistance among *Acinetobacter baumannii* isolates is also quite high in the countries of the Arab League, ranging from 36 to 100 percent (Moghnieh et al. 2018). The epidemiology of serious hospital-acquired infections has been influenced by the rising prevalence of antimicrobial resistance among *Acinetobacter baumannii* isolates. One systematic review showed that carbapenem-resistant and multidrug-resistant *Acinetobacter baumannii* accounted for 65 and 59 percent, respectively, of all hospital-acquired infections among intensive care unit patients in Southeast Asia (Teerawattanapong et al. 2018).

Polymyxin B and polymyxin E (Colistin) are the most commonly used agents for *Acinetobacter* isolates resistant to first-line agents. There are no randomized trials addressing their efficacy, largely because they are reserved for use in the setting of highly resistant organisms. Colistin had some success for the treatment of *Acinetobacter* pneumonia, bacteraemia, and meningitis (Garnacho-Montero et al. 2003, Levin et al. 1999). Among nine studies (178 patients) that did

not include a comparator treatment, the pooled clinical response rate for intravenous colistin was 66%. However, one small series of 20 cases of nosocomial pneumonia that was not included in the analysis reported a success rate of only 25 percent (Levin et al. 1999). Nephrotoxicity is the most notorious adverse effect associated with systemic colistin and has been reported in up to 36 percent of patients (Falagas et al. 2006). Neurotoxicity is another important side effect but consists mainly of paraesthesia and is relatively uncommon. Colistin dosing depends on the available preparation and should be adjusted in patients with impaired renal function. Polymyxin B is associated with lower rates of nephrotoxicity than Colistin.

Sulbactam, a beta lactamase inhibitor, has shown to have good in vitro activity against *Acinetobacter* species (Urban et al. 1993). In HUSM, sulbactam is available in combination form namely as ampicillin-sulbactam and cefoperazone-sulbactam. Several studies have suggested that sulbactam might be effective in *Acinetobacter* infection. For example, high dose ampicillin-sulbactam was evaluated as an alternative treatment of late onset ventilator associated pneumonia from multidrug resistant *Acinetobacter baumannii* (Betrosian et al. 2007). The aim of the study was to evaluate the efficacy and safety of two high dose treatment regiments of ampicillin-sulbactam for multi-drug resistant *Acinetobacter baumannii* VAP. It was a randomised prospective trial in Hippokration General Hospital in Athens consisted of 27 patients. Mortality rates did not differ significantly between the two groups. No major adverse reactions were recorded. The conclusion that the study supported the use of high dose regimen of ampicillin-sulbactam for MDR *Acinetobacter baumannii* VAP. However due to the small sample size, the result of the study was not statistically strong.

A retrospective case series study in Korea evaluated the efficacy of high dose sulbactam treatment for ventilator associated pneumonia caused by carbapenem resistant *Acinetobacter baumannii* (Jeong et al. 2016). The conclusion of the study was that high dose sulbactam could be effective for the treatment of CRAB ventilator associated pneumonia. However early clinical failure was

common and is associated with a higher mortality with the treatment. The sample size was small and the study was not a randomised clinical trial.

In 2013, a systematic review and meta-analysis of sulbactam based therapy for *Acinetobacter baumannii* infection was published (Chu et al. 2013). This meta-analysis consisted of four studies three of which were retrospective while one was prospective. Treatment with sulbactam was compared to treatment with other classes of antibiotics. The results suggested that sulbactam-based therapy may be efficacious to alternative antimicrobial therapy for the treatment of *Acinetobacter* infection. However, only a very small number of trials were included and none of the trial were randomised trials. Furthermore the number of participants in the studies was relatively small and thus the power of the study was not strong enough.

Another study compared the efficacy of ampicillin/sulbactam and Colistin in the treatment of multidrug resistant *Acinetobacter baumannii* ventilator associated pneumonia (Betrosian et al. 2008). This was a prospective cohort study in 28 adults in the intensive care units in Hippokration General Hospital in Athens. The conclusion was that Colistin and high dose ampicillin/sulbactam were comparably safe and effective treatments for critically ill patients with MDR *Acinetobacter baumannii* VAP. However, the sample size of this study was small and the statistical power of this study was weak.

In addition, one retrospective study compared ampicillin/sulbactam with polymyxin for the treatment of infections caused by carbapenem- resistant *Acinetobacter* species (Oliveira et al. 2008). The study consisted of a total of 190 patients and was carried out in 2 large teaching hospitals in Brazil. The findings of the study was that ampicillin/sulbactam appeared to be more efficacious than polymyxin, which was an independent factor associated with mortality during treatment. However, the polymyxin group consisted of significantly older patients, more frequently submitted to surgical procedures and had more patients with cancer.

Furthermore, a 2003 retrospective study consisted of treating 40 MDR *Acinetobacter baumannii* infected patients with intravenous ampicillin/sulbactam (Levin et al. 2003). The median dose of ampicillin/sulbactam was 6g/3g. There were no observed adverse effects and that study indicated that ampicillin/sulbactam might be a good and safe therapeutic option to treat severe *Acinetobacter baumannii* nosocomial infections. However the study was not a randomised clinical trial.

In 1998, a prospective study was published whereby sulbactam was evaluated in 40 patients with non-life threatening multiresistant *Acinetobacter baumannii* infection in the Hospital de Bellvitge in Barcelona (Corbella et al, 1998). 18 patients received intravenous sulbactam alone versus 24 who received intravenous ampicillin-sulbactam. The results of the study suggested that sulbactam might prove effective for non-life threatening *Acinetobacter baumannii* infections. However, its role in the treatment of severe infections was unknown.

These studies have showed promising results of sulbactam based therapy in *Acinetobacter* infection. However, to our knowledge, no similar study was carried out in Malaysia before. We wanted to assess the outcomes of treating *Acinetobacter* infection in our population with sulbactam. The hypothesis was that sulbactam was as effective as polymyxin B in treating *Acinetobacter* infection. Thus, this study's results would provide a better insight on the accuracy of the hypothesis.

CHAPTER 2

OBJECTIVES OF THE STUDY

GENERAL OBJECTIVE

- To study the outcomes of patients with *Acinetobacter* infection.

SPECIFIC OBJECTIVES

1. To determine the proportion of patients with *Acinetobacter* infection treated with polymyxin versus non polymyxin based treatment.

2. To determine the association between polymyxin and non polymyxin based therapy among patients with *Acinetobacter* infection in terms of health outcomes: success versus failure.

CHAPTER 3

MANUSCRIPT

TITLE

Treatment outcomes of patients with *Acinetobacter* infection; comparison between polymyxin versus non polymyxin based therapy

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ABSTRACT

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INTRODUCTION

Acinetobacter species is a recognised pathogen implicated in a wide range of nosocomial infections. Its growing resistance to almost all commercially available antibiotics is of major concern. Till date, there has a lack of randomised clinical trials to evaluate the best antimicrobial regimen for treating *Acinetobacter* infections. In clinical practice, Polymyxin B and Colistin (Polymyxin E) are being used. They have good in vitro activity against many gram negative bacilli including *Acinetobacter* species. The major adverse effects are nephrotoxicity, neurotoxicity and neuromuscular blockade (Evans et al. 1999, Horton et al. 1982). At the Hospital Universiti Sains Malaysia, Polymyxin B is the current available therapy for the *Acinetobacter* infection. It is a relatively expensive treatment and therefore its use is strictly regulated. Sulbactam, a beta lactamase inhibitor, has shown to have good in vitro activity against *Acinetobacter* species (Urban et al, 1993). Some studies have suggested that sulbactam might be effective in *Acinetobacter* infection (Betrosian et al. 2007, Betrosian et al. 2008, Chu et al. 2013, Corbella et al. 1998, Jeong et al. 2016, Levin et al. 2003, Oliveira et al. 2008). At our centre, sulbactam is available in combination forms namely as ampicillin-sulbactam and cefoperazone-sulbactam. Unasyn® is sulbactam combined with ampicillin in a fixed 2:1 ratio while sulperazone® is sulbactam combined with cefoperazone in a ratio of 1:1. Sulbactam is a well-tolerated drug with the main adverse effects being pain at the site of injection, diarrhoea and rash. In addition, the cost of the treatment with sulbactam is affordable to the general public. The aim of the study was to compare the efficacy of sulbactam regime to polymyxin B in the treatment *Acinetobacter* infection.

METHODOLOGY

Study population

This was a retrospective study of case records over one year period (1st January 2018 to 31st December 2018) at the Hospital Universiti Sains Malaysia (HUSM). HUSM is a tertiary care teaching hospital located in the north east state of Kelantan in Malaysia. The enrolled cases were hospitalised patients who were at least 18 years old with clinical evidence of infection and with isolation of *Acinetobacter* species from a specific culture site. Those patients who were already on treatment with either polymyxin B or sulbactam for other concomitant infection, on the day of isolation of *Acinetobacter*, were excluded. The demographic, clinical and laboratory data from the patient's file were collected. The study cohort was divided into two groups namely the polymyxin group and the nonpolymyxin group. Each infection was defined using some specific criteria as mentioned below.

For instance, pneumonia was defined as patient having a new or progressive radiographic parenchymal lung infiltrate with some signs that the infiltrate was infectious in origin. This required the presence of at least 2 of the following signs: temperature alteration (less than 36°C or at least 38.3°C), a white blood cell count less than 5000 cells/mm^3 or more than 10,000 cells/mm^3, or purulent-appearing sputum or endotracheal aspirate. Hospital Acquired Pneumonia (HAP) referred to the development of parenchymal lung infection after at least 48 hours of hospitalisation. On the other hand, if the infection developed after the patient underwent intubation and received mechanical ventilation for at least 48 hours, the condition was termed Ventilator Associated Pneumonia (VAP).

Bloodstream Infection included the primary, secondary and central line associated bloodstream infections.

- Primary bloodstream infection was defined as a laboratory confirmed bloodstream infection that was not secondary to an infection at another body site.
- Secondary bloodstream infection was defined as a bloodstream infection that was thought to be seeded from a site-specific infection at another body site.
- Central line-associated bloodstream infection was defined as a laboratory confirmed bloodstream infection where an eligible bloodstream infection organism was identified and an eligible central line was present on the laboratory confirmed bloodstream infection day of event or the day before.

Surgical site infection occurred within 30 days of surgery and involved any part of the body deeper than the fascia/muscle layers that was opened or manipulated during the operative procedure. The patient had at least one of the following:

- purulent drainage from a drain that is placed into the organ/space
- organism(s) identified from fluid or tissue in the organ/space by a culture
- an abscess or other evidence of infection involving the organ/space that is detected on gross anatomical or histopathologic exam, or imaging test evidence suggestive of infection.

Urinary tract infection was defined as patient having at least one of the following signs or symptoms: fever (temperature of at least 38.0°C), suprapubic tenderness, costovertebral angle pain or tenderness, urinary urgency, urinary frequency or dysuria. In addition, the patient's voided urine should yield a culture of at least 10^5 CFU/ml of not more than 2 species of microorganisms.

Meningitis was defined as patient having at least two of the following: fever (temperature of at least 38.0°C) or meningeal sign(s), cranial nerve sign(s) with

- Organism identified from cerebrospinal fluid (CSF) by a culture
- organism seen on Gram stain of CSF
- increased white cells, elevated protein, and decreased glucose in CSF (per reporting laboratory's reference range)

National Early Warning Score 2 (NEWS2) is a scoring system used for the assessment and response to acute illness. Six parameters form the basis of the scoring system: respiratory rate, oxygen saturation, systolic blood pressure, heart rate, level of consciousness and temperature. The NEWS2 holds a separate section for scoring oxygen saturations in patients with chronic respiratory failure, in whom oxygen saturation of 88-92% are recommended. The NEWS2 score calculated on the day of initiation of polymyxin, ampicillin-sulbactam and cefoperazone-sulbactam was taken into account in this study.

Table 1: NEWS2 scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

(NEWS2 Standardising the assessment on acute illness severity in the NHS, Royal College of Physicians)

LOW score: an aggregate NEWS2 score of 1–4

MEDIUM score: an aggregate NEWS2 score of 5 or 6.

HIGH score: an aggregate NEWS2 score of 7 or more.

Definition of Outcome Events

The treatment efficacy was assessed on day 5 of treatment. It comprised of 3 outcomes: microbiological response, clinical response and 30 days in patient mortality.

The clinical response was defined as

- Success if signs and symptoms improved and/or a decrease of at least 50% on initial CRP at day 5 of treatment.
- Failure if symptoms and signs persisted or worsened at day 5 of treatment.

The microbiological response was defined as

- Success if there was eradication of *Acinetobacter* species from culture at day 5 of treatment.
- Failure if persistence of *Acinetobacter* species at day 5 of treatment.

30 days in patient mortality was defined as any death of *Acinetobacter* infected patients within 30 days of starting treatment in hospital setting.

Statistical Analysis

Data was entered and analysed using SPSS version 24. The results were expressed in terms of numbers and percentages or mean and standard deviation. The categorical variables were tested using the chi square test while the student's t-test was used for continuous variables. A p-value of <0.05 was considered significant. In addition, logistic regression analysis was carried out to evaluate the potential independent risk factors for mortality.

Ethical Issue

This study was conducted in accordance with the principles laid by the 18th World Medical Assembly (Helsinky, 1964), and all subsequent amendments. It was approved by the Human Research Ethics Committee of USM (JEPeM) on the 8th April 2019 (Reference number: **USM/JEPeM/ 19010069**). The official authorisation to access patients' folders was granted from the Director of HUSM. The Infectious Control and Epidemiology Unit (UKJEH) of HUSM was contacted in order to get the list of patients with culture positive for *Acinetobacter* for the intended time period. The patients' personal identification and clinical data were confidential. No conflict of interest was involved in this study and no payment was given or received from any company or organization. All of the information obtained from the medical records was recorded in a password-protected computer folder to prevent any intentional or unintentional breach of patient's confidentiality.

RESULTS

A total of two hundred and eighteen cases were reviewed. Among these cases, one hundred and forty were either contaminants or colonisers. Only seventy-eight were *Acinetobacter* infections. Six of them were excluded as they were treated with a different antibiotic (piperacillin-tazobactam). Purposive sampling was carried out. Thirty-four received polymyxin treatment, twenty-four received ampicillin-sulbactam and fourteen received cefoperazone-sulbactam (Table 2). Thus, the nonpolymyxin group had a total of thirty-eight patients (52.8%).

The initial sample size calculated was one hundred and forty. However, at the end of the study, only seventy-two cases were obtained. The exact prevalence of acinetobacter infection in HUSM was unknown, so it was difficult to determine the proportion of *Acinetobacter* infection beforehand. As this was a retrospective study and we were limited in time, we could not afford to search for more cases in order to meet the calculated sample size. Furthermore, there were twenty case notes which could not be traced during the study period. .

The characteristics of the study population are summarised in the Table 3. There were forty-six (63.9%) males and the mean age was 55.0 years old. Forty patients (55.6%) were admitted to ICU while fifteen (20.8%) were admitted in HDU and seventeen (23.6%) were admitted to general wards. Four (5.6%) had end stage renal disease while three (3.4%) had chronic liver disease. Thirty-one (43.1%) were diabetics while eleven (15.3%) had a specific underlying malignant condition. The mean NEWS2 Score of the population was 6.8. Sixty-six (91.7%) were infected with multidrug resistant *Acinetobacter* species.

The majority of the *Acinetobacter* infections was ventilator associated pneumonia, with twenty-four (70.6%) in the polymyxin group versus twenty-one (55.3%) in the nonpolymyxin group (Table 4). Five (14.7%) and nine (23.7%) in the polymyxin and nonpolymyxin group respectively had bloodstream infection. There was only one case (2.9%) of meningitis treated with polymyxin

while on the other hand there was only one case (2.6%) of urinary tract infection treated in the nonpolymyxin group. Two (5.9%) hospital acquired pneumonia were in the polymyxin group while three (7.9%) hospital acquired pneumonia cases were in the nonpolymyxin group.

In the polymyxin group, the mean age was 50.6 years old compared to 58.9 years old in the nonpolymyxin group (Table 5). The mean NEWS2 score of the polymyxin group was higher compared to that of the nonpolymyxin group (8.1 vs. 5.6). Seventeen (50%) in the polymyxin group had septic shock compared to three (7.9%) in the nonpolymyxin group. Thirty-three cases (97.1%) of multidrug resistant acinetobacter infection were present in the polymyxin group compared to thirty-three (86.8%) in the other group. There were more diabetics with twenty (52.6%) in the nonpolymyxin group versus eleven (32.4%) in the polymyxin group. Two patients (5.9%) had end stage renal disease in the polymyxin group and there were two patients (5.3%) in the nonpolymyxin group as well. Chronic liver disease was present in two patients (5.9%) in the polymyxin group and one patient (2.6%) in the nonpolymyxin group. Six (17.6%) had a specific underlying malignant condition in the polymyxin group and five (13.2%) in the nonpolymyxin group. Twenty-three (67.6%) were males in the polymyxin group and similarly there were twenty-three (60.5%) males in the nonpolymyxin group. Twenty-four (70.6%) in the polymyxin group required ICU admission compared to sixteen (42.1%) in the nonpolymyxin group. The mean number of days between isolation of *Acinetobacter* and start of treatment in both group is almost similar: 1.79 days in the polymyxin group vs. 1.42 days in the nonpolymyxin group.

Twenty-four (63.2%) from the nonpolymyxin group achieved clinical success while in the polymyxin group only thirteen (38.2%) achieved clinical success (Table 6). Twenty-six (68.4%) achieved microbiological success in the nonpolymyxin group versus eighteen (52.9%) in the polymyxin group. Mortality was lower in the nonpolymyxin group with seventeen deaths (44.7%) compared to twenty-three deaths (67.6%) in the polymyxin group.

The logistic regression analysis results for the 30-day in patient mortality is shown in Table 7. Based on p-value <0.25, the following variables were selected to multiple logistic regression analysis: NEWS2 score, male gender, malignancy, septic shock, polymyxin group, and microbiological outcome.

By using method Forward LR for variable selection, variable microbiological outcome remained in the model for analysis multiple logistic regression (Table 8). Thus, microbiological failure was significantly associated with the 30-days in patient mortality.

DISCUSSION

Acinetobacter is known to be one of the most frequent infective organisms in intensive care units. One study showed that 54.9% of *Acinetobacter* species isolates were obtained from ICUs, 36.7% and 8.4% from the medical and surgical units respectively (Uwingabiye et al. 2016). Another study noted that *Acinetobacter baumannii* was more frequently associated with infection among patients in the ICU (63.9%) compared to patients admitted to medical (52.8%) and to surgical wards (52.9%) (Villar et al. 2014). Similarly, our study found a predominance of *Acinetobacter* infections in intensive care unit. Forty patients (55.6%) were from ICU while fifteen (20.8%) were from HDU and seventeen (23.6%) were from general wards.

The majority of the *Acinetobacter* infections was ventilator associated pneumonia, with twenty-four patients (70.6%) in the polymyxin group versus twenty-one (55.3%) in the nonpolymyxin group. Five (14.7%) and nine (23.7%) in the polymyxin and nonpolymyxin group respectively had bloodstream infection. Our study was in concordance with other studies whereby VAP was proved to be the most common *Acinetobacter* infection. For instance, one study showed that VAP accounted for 73.8% of "*Acinetobacter baumannii*" infection (Duszynska et al. 2018) while

another study concluded that pneumonia was the most common site of "*Acinetobacter baumannii*" infection (53.1%) (Castilho et al. 2017).

There was one case (2.9%) of multidrug resistant *Acinetobacter* meningitis in our study which was detected in the CSF of a 22-year-old patient who underwent neurosurgical intervention for pineal gland tumour. The patient was treated with polymyxin but unfortunately, the treatment was unsuccessful and the patient passed away in ICU. This case outlines the difficulty in treating *Acinetobacter* meningitis and highlights its associated high mortality rate. Chen et al. (2005) noted a 30% mortality rate among patients with *Acinetobacter* meningitis while Rodriguez et al. (2008) noted a mortality rate of 33.3% in patients with nosocomial neurosurgical meningitis.

It has been a common practice at our hospital to use polymyxin for the younger and more severely ill patient infected with *Acinetobacter* in order to maximise their prospect of cure and survival. This was evidenced by our data results that showed a lower mean age in the polymyxin group (58.9 years vs. 50.6 years) but with a higher percentage of septic shock (50% vs. 7.9%).

43.1% of the study population were diabetics. Even though there were more diabetics in the nonpolymyxin group than in the polymyxin group (52.6% vs. 32.4%), our study did not show any relationship between diabetes and the outcomes in the two groups. Furthermore, diabetes did not have any significant impact on the mortality. This is in contrast to the study led by Leung et al. (2019) which found that mortality was higher in diabetic patient with *Acinetobacter* infection.

In terms of outcomes, the nonpolymyxin group fared better compared to the polymyxin group. Twenty-four patients (63.2%) from nonpolymyxin group achieved clinical success while in the polymyxin group only thirteen (38.2%) achieved clinical success. This success achieved statistical significance ($p=0.035$). Levin et al. (2003) studied twelve patients with ampicillin-sulbactam and the results showed 67.5% had clinical improvement. Corbella and al. (1998) treated forty-two cases of non-life threatening *Acinetobacter* infection with sulbactam and noted a clinical

improvement in 92.9%. Thus, our clinical outcome is consistent with these studies that used sulbactam as an alternative treatment in *Acinetobacter* infection.

Twenty-six patients (68.4%) achieved microbiological success in the nonpolymyxin group versus 18 (52.9%) in the polymyxin group. Of note, eight (23.5%) from polymyxin group and five (13.2%) from nonpolymyxin group did not have repeated culture samples. Thus, the microbiological outcomes could not be assessed in these thirteen cases. This could partly explain why the microbiological outcome did not achieve statistical significance. Nevertheless, this result showed a better microbiological outcome with the nonpolymyxin therapy. This is in keeping with a study which found that ampicillin-sulbactam treated carbapenem resistant *Acinetobacter* had a cure/improvement rate of 70% (Oliveira et al, 2008). Another study showed comparable bacteriologic success in patients infected with multidrug resistant *Acinetobacter baumannii* treated with ampicillin-sulbactam (61.5%) (Betrosian et al. 2008).

The overall mortality in this study was forty patients (55.6%). Likewise, the seven year experience of Kanafani et al. (2018) on multidrug resistant *Acinetobacter* noted a mortality rate ranging from 52% to 66% among the infected patients. Furthermore, a prospective study by Sileem et al. (2017) showed that the mortality in patients who developed nosocomial *Acinetobacter* infection was 50%.

The nonpolymyxin group had better mortality outcomes with lesser deaths: seventeen (42.5%) compared to twenty-three (67.6%) in the polymyxin group. Although the result was not significant ($p = 0.051$), the trend in mortality outcome was similar to that observed in both microbiological and clinical outcomes. A possible explanation for lesser deaths is the severity of the illness in the polymyxin group. The NEWS2 score was higher in the polymyxin group (8.12 vs. 5.55) and there were more patients in the polymyxin group admitted to the ICU (70.6% vs. 42.1%). In addition 50% of patients treated with polymyxin were in septic shock compared to only 7.9% treated with nonpolymyxin.

The univariate analysis performed for the 30 days in patient mortality showed the following variables as independent risk factors for mortality: higher NEWS2 score, male gender, malignancy, septic shock, polymyxin group and microbiological failure. Worsening of any infection is usually accompanied by multi-organ failure and subsequently death. Hence, the association with higher NEWS2 score and septic shock with mortality is plausible.

An interesting finding of this study is that the gender male was associated with mortality. One study reported that *Acinetobacter baumannii* infection was more frequent in males (Drault et al. 2001). This male predominance was explained by the fact that *Acinetobacter baumannii* is often associated with underlying conditions like smoking, alcohol, diabetes and pneumopathies. In contrast, Uwingabiye et al. (2016) also showed male predominance in their study of *Acinetobacter* infection but no reason was justified. Within the scope of our study, a reasonable explanation for the male predominance was that there were almost twice number of males with malignancy compared to females. 36.4% of malignancy cases were found in females compared to 73.6% in males.

Malignancy is obviously an independent risk factor of mortality. In these cases of *Acinetobacter* infection with malignancy, it is difficult to differentiate between the deaths attributable to *Acinetobacter* infection versus those attributable to the underlying malignancy.

Following the univariate analysis, a multivariate analysis was performed for the 30-day in patient mortality. The result was that microbiological failure was found to be the only independent factor significantly associated with mortality in this study.

LIMITATION AND RECOMMENDATION

Our study does have its limitations. One of them is that it is a retrospective study. More severely ill patients were noted in the polymyxin group. Another limitation is the small sample size of our

study. This might have contributed for not reaching statistical significance in the microbiological and mortality outcomes. Nevertheless, the number of patients infected with *Acinetobacter* is usually limited and therefore, our results should not be underestimated. Microbiological failure, which was determined five days after start of sulbactam treatment, was significantly associated with 30 days mortality. Since microbiological failure is a risk factor of mortality, we advise for immediate change of antibiotics once microbiological failure is detected.

CONCLUSION

To our knowledge, this is the first study in Malaysia comparing polymyxin versus sulbactam based therapy in *Acinetobacter* infection. The most important finding of our study is that sulbactam appears to have a better efficacy compared to polymyxin in treating *Acinetobacter* infection.

APPENDICES

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TABLES

Table 2: Number of patients with specific antibiotics treatment

Group	Frequency	%
Polymyxin	34	47.2
Ampicillin-sulbactam	24	33.3
Cefoperazone-sulbactam	14	19.5
Total	72	100.0

Table 3: Characteristics of patients with *Acinetobacter* infections

Variables	Mean (SD)	Frequency (%)
Age	55.0 (15.8)	
NEWS2 Score	6.8 (2.9)	
ICU admission		40 (55.6)
Male		46 (63.9)
End stage renal disease		4 (5.6)
Chronic liver disease		3 (4.2)
Diabetes		31 (43.1)
Malignancy		11 (15.3)
MDRAI		66 (91.7)
Septic shock		20 (27.8)

Table 4: Types of *Acinetobacter* infections in both polymyxin and non polymyxin group

Infection	**Polymyxin group** **Number of cases (%)**	**Non polymyxin group** **Number of cases (%)**
Ventilator associated pneumonia	24 (70.6)	21 (55.3)
Bloodstream infection	5 (14.7)	9 (23.7)
Surgical site infection	2 (5.9)	4 (10.5)
Hospital acquired pneumonia	2 (5.9)	3 (7.9)
Meningitis	1 (2.9)	0 (0)
Urinary tract infection	0 (0)	1 (2.6)
Total	34 (100)	38 (100)

p- value = 0.328

Table 5: Characteristics of patients in polymyxin vs. non polymyxin group

Variables	**Polymyxin Group** **N = 34**	**Non polymyxin group** **N = 38**	**p-value**
Age Mean (SD)	50.6 (15.9)	58.9 (14.7)	0.025
NEWS2 Mean (SD)	8.1 (2.7)	5.6 (2.5)	0.000
ICU admission	24 (70.6%)	16 (42.1%)	0.003
Male	23 (67.6%)	23 (60.5%)	0.530
End stage renal disease	2 (5.9%)	2 (5.3%)	0.909
Chronic liver disease	2 (5.9%)	1 (2.6%)	0.491
Diabetes	11 (32.4%)	20 (52.6%)	0.083
Malignancy	6 (17.6%)	5 (13.2%)	0.597
Septic shock	17 (50%)	3 (7.9%)	0.000
MDRAI	33 (97.1%)	33 (86.8%)	0.117
Days Mean (SD)	1.79 (1.74)	1.42 (1.73)	0.366

Table 6: Clinical, microbiological and mortality outcomes in the study groups

	Polymyxin group **N = 34**	**Nonpolymyxin group** **N = 38**	**p-value**
Clinical			
Success	13 (38.2%)	24 (63.2%)	0.035
Failure	21 (61.8%)	14 (36.8%)	
Microbiological			
Success	18 (52.9%)	26 (68.4%)	0.403
Failure	8 (23.5%)	7 (18.4%)	
30 days Mortality			
Alive	11 (32.4%)	21 (55.3%)	0.051
Death	23 (67.6%)	17 (44.7%)	

Table 7: Simple logistic regression for 30 days in patient mortality

Variables	Crude OR (95% CI)	p-value
Age	1.01 (0.98,1.04)	0.490
NEWS Score	0.95 (0.72, 1.24)	0.691
ICU admission	0.82 (0.25,2.72)	0.739
Male	1.81 (0.69,4.80)	0.230
End stage renal disease	2.51 (0.25,25.40)	0.435
Chronic liver disease	0.39 (0.03,4.44)	0.444
Diabetes	0.95 (0.37,2.43)	0.915
Malignancy	2.42 (0.59,9.99)	0.223
Non MDRAI	0.37 (0.07,2.16)	0.268
Septic shock	3.24 (1.03,10.22)	0.045
Polymyxin	0.39 (0.15,1.01)	0.053
Microbiological outcome	0.09 (0.02,0.44)	0.003

Table 8: Multiple logistic regression analysis for 30 day in patient mortality

Variables	Adjusted OR (95% CI)	p-value
Microbiological outcome		
Failure	1	
Success	0.09(0.02,0.44)	0.003

STUDY PROTOCOL

Research Title

Treatment outcomes of patients with *Acinetobacter* infection; comparison between polymyxin versus non polymyxin based therapy.

Candidate

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Introduction

Acinetobacter species is a recognised pathogen implicated in a wide range of clinical diseases such as blood stream infection, pneumonia, surgical site infection, meningitis, urinary tract infection, intravascular devices and implant-related infections. Its growing resistance to almost all commercially available antibiotics (carbapenem, cephalosporin, aminoglycoside, fluoroquinolone) is causing a severe treatment problem. Currently, there are limited therapeutic options are available against these infections. Polymyxin B and polymyxin E (Colistin) are the available therapies for the *Acinetobacter* infection. At the Hospital universiti Sains Malaysia, polymyxin B is the current drug used for *Acinetobacter* infections. However, there are major adverse effects associated with it as nephrotoxicity, neurotoxicity and neuromuscular blockade. Sulbactam, a beta lactamase inhibitor has shown to have good in vitro activity against

Acinetobacter species. Some studies have suggested that sulbactam might be effective in *Acinetobacter* infection. At our centre, sulbactam is available in combination with ampicillin in a fixed ratio 2:1 known as Ampicillin-sulbactam. It is a well-tolerated drug with the main adverse effects being pain at the site of injection, diarrhoea and rash. The aim of the study is to assess the clinical efficacy of high dose regimen ampicillin-sulbactam compared to polymyxin B in *Acinetobacter* infection.

Problem statement & Study rationale

To compare the efficacy of sulbactam-ampicillin versus polymyxin B in *Acinetobacter* infection.

To reduce the usage of polymyxin B as well as to provide an alternative to polymyxin B.

Research Question(s)

Is sulbactam-ampicillin therapy as effective as polymyxin B in the treatment of *Acinetobacter* infection?

Objective

General:

To study the health outcomes of patients infected with *Acinetobacter* infection

Specific

3. To determine the proportion of patients with *Acinetobacter* infection treated with polymyxin versus non polymyxin based treatment.
4. To determine the association between polymyxin and non polymyxin based therapy among patients with *Acinetobacter* infection in terms of health outcomes: success versus failure

Literature review

In 2006, Betrosian et al evaluated high dose ampicillin-sulbactam as an alternative treatment of late onset ventilator associated pneumonia from multidrug resistant *Acinetobacter baumannii*. The aim of the study was to evaluate the efficacy and safety of 2 high dose treatment regiments of ampicillin-sulbactam for multi-drug resistant *Acinetobacter baumannii* VAP. It was a randomised prospective trial in Hippokration General Hospital in Athens consisted of 27 patients. Mortality rates did not differ significantly between the two groups. No major adverse reactions were recorded. The conclusion that the study supported the use of high dose regimen of ampicillin-sulbactam for MDR *Acinetobacter baumannii* VAP. However due to the small sample size, the result of the study was not statistically strong.

A retrospective study lead by Oliveira et al in 2007 compared ampicillin/sulbactam with polymyxin for the treatment of infections caused by carbapenem- resistant *Acinetobacter* species. The study consisted of a total of 190 patients and was carried out in 2 large teaching hospitals in Brazil. The findings of the study was that ampicillin/sulbactam appeared to be more efficacious than polymyxin, which was an independent factor associated with mortality during treatment. However, the polymyxin group consisted of significantly older patients, more frequently submitted to surgical procedures and had more patients with cancer.

Another study by Betrosian et al in 2008 compared the efficacy of ampicillin/sulbactam and Colistin in the treatment of multidrug resistant *Acinetobacter baumannii* ventilator associated pneumonia. This was a prospective cohort study in 28 adults in the intensive care units in Hippokration General Hospital in Athens. The conclusion was that Colistin and high dose ampicillin/sulbactam were comparably safe and effective treatments for critically ill patients with MDR *Acinetobacter baumannii* VAP. However, the sample size of this study was small and the statistical power of this study was weak.

Levin et al lead a retrospective study in 2002 at the University Sao Paolo, Brazil. It consisted of forty consecutive patients with nosocomial infection caused by MDR *Acinetobacter baumannii*, who were treated with intravenous ampicillin/sulbactam. The median dose of ampicillin/sulbactam was 6g/3g. There were no observed adverse effects and that study indicated that ampicillin/sulbactam might be a good and safe therapeutic option to treat severe *Acinetobacter* baumaanii nosocomial infections. However the study was not a randomised clinical trial.

In 1998 Corbella et al published a prospective study whereby sulbactam was evaluated in 42 patients with non-life threatening multiresistant *Acinetobacter baumannii* infection in the Hospital de Bellvitge in Barcelona. 18 patients received intravenous sulbactam alone versus 24 who received intravenous ampicillin-sulbactam. The results of the study suggested that sulbactam might prove effective for non-life threatening *Acinetobacter baumannii* infections. However its role in the treatment of severe infections was unknown.

A retrospective case series study was conducted by In Beom Jeong et al in 2016 in Korea evaluated the efficacy of high dose sulbactam treatment for ventilator associated pneumonia caused by carbapenem resistant *Acinetobacter baumannii* (CRAB). The conclusion of the study was that high dose sulbactam could be effective for the treatment of CRAB ventilator associated pneumonia. However early clinical failure was common and is associated with a higher mortality with the treatment. The sample size was small and the study was not a randomised clinical trial.

In 2012 Haiqing et al published a systematic review and meta-analysis of sulbactam based therapy for *Acinetobacter baumannii* infection. The meta-analysis consisted of four studies three of which were retrospective while one was prospective. Treatment with sulbactam was compared to treatment with other classes of antibiotics. The results suggested that sulbactam-based therapy may be efficacious to alternative antimicrobial therapy for the treatment of *Acinetobacter*

infection. However, only a very small number of trials were included and none of the trial were randomised trials. Furthermore the number of participants in the studies were relatively small and thus the power of the study was not strong enough.

Justification of study:

- To provide a baseline study for future research in HUSM involving *Acinetobacter*.
- To assess the efficacy of sulbactam in *Acinetobacter* infections and compare to other international studies done previously.

Research design

Retrospective study over 1 year (1st January 2018 to 31st December 2018)

Study area

Hospital Universiti Sains Malaysia

Study population

Adult patients admitted to the ward, Intensive Care Unit or High dependency Unit of Hospital Universiti Sains Malaysia.

Inclusion criteria

- Evidence of infection
- Isolation of *Acinetobacter* from culture.
- Age of at least 18 years old

Exclusion criteria

- Patients already on treatment with either polymyxin B or sulbactam-ampicillin for concomitant infections on the day of diagnosis of *Acinetobacter* infection will be excluded.

Sample size estimation

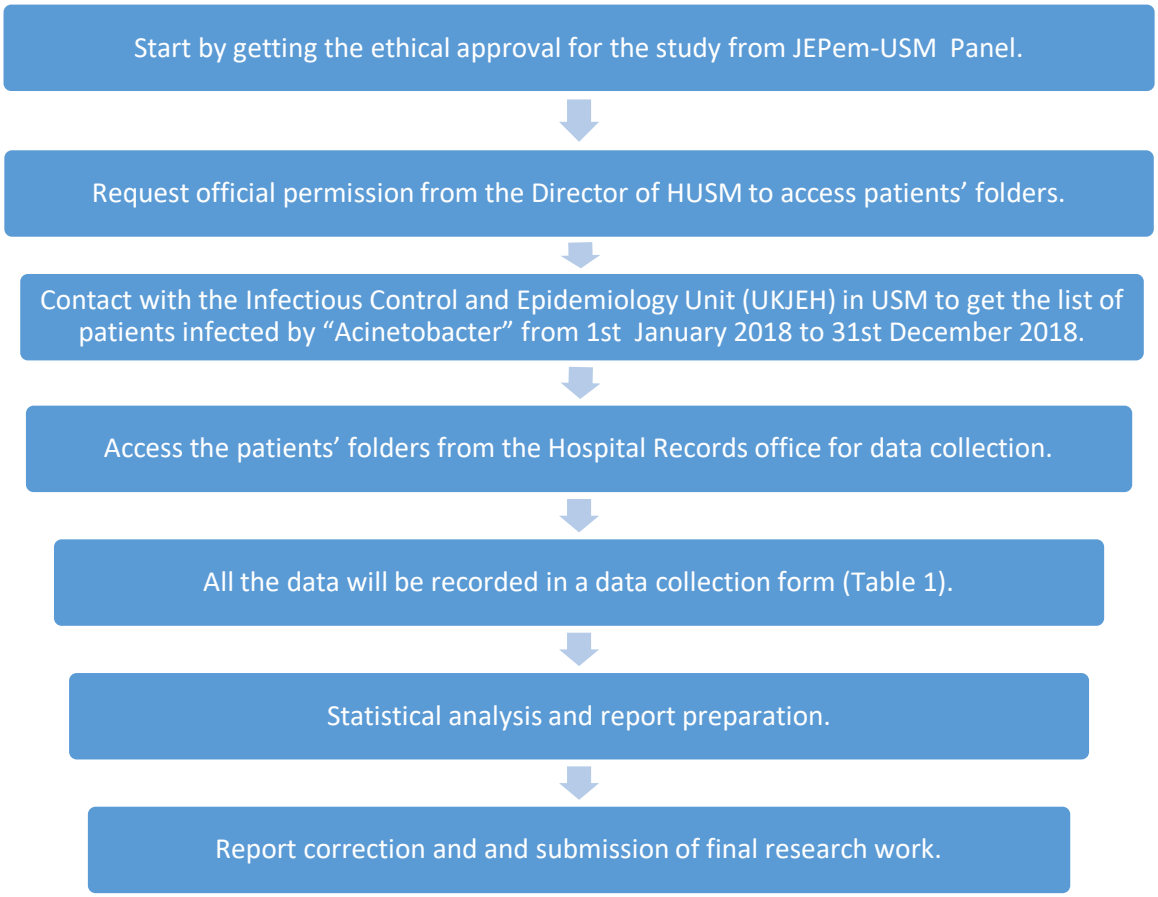
Sample size will be calculated by using 2 proportion formula as shown below.

VARIABLE	**LITERATURE REVIEW**	**Alpha**	**Power**	**P0**	**P1**	**Subjects in each group**	**Total**
Patient related factors:							
Success	Betrosian et al. Efficacy and safety of high dose ampicillin/sulbactam vs. Colistin as monotherapy for treatment of multidrug resistant *Acinetobacter baumannii* ventilator-associated pneumonia	0.05	0.7	0.6	0.8	64	128

With anticipation of drop outs and incomplete data, the sample size was estimated to a total of 140 with 70 subjects in each arm.

According to UKJEH (Infectious Control and Epidemiology Unit), around 490 cases of *Acinetobacter* were detected in Hospital Universiti Sains Malaysia in the year 2018. We are confident to obtain 70 cases in each arm of the study among those 490 cases.

Study flowchart



Data analysis

Data will be entered and analysed using SPSS version *24*. Descriptive statistics will be used to summarise the socio-demographic characteristics of subjects. Numerical data will be presented as mean (SD) or median (IQR) based on their normality distribution. Categorical data will be presented as frequency (percentage).

JEPeM-USM Review Panel and regulatory authorities may review study data if required.

Gantt chart

Research activity in 2019	*January*	*February*	*March*	*April*	*May*	*June*	*July*
Dissertation proposal and ethics approval							
Data collection							
Data analysis and interpretation							
Submission of draft and revision							

Budget proposal:

Not applicable

Ethical consideration(s):

1. Subject vulnerability

Not applicable

2. Declaration of absence of conflict of interest

The investigator has no conflict of interest in connection to this study.

3. Privacy and confidentiality

All forms are anonymous and will be entered into SPSS software. Only research team members can access the data. Data will be presented as grouped data and will not identify the responders individually.

4. Community sensitivities and benefits

Not applicable

5. Honorarium and incentives

The investigator has not sought, accepted or attempted to obtain any advantage, financial or in any other forms in relation to this study.

The investigator has not granted any advantage, financial or in any other forms to any party in relation to this study.

6. Other ethical review board approval [if applicable]

Not applicable

Table 9: Data collection form

Date of admission	/ /					
Unit admitted	ICU	HDU	Ward			
Gender	Male	Female				
Age						
NEWS (2) Score						
Comorbid						
ESRF	Yes	No				
Diabetes	Yes	No				
Hep B/C/ Liver cirrhosis	Yes	No				
Malignancy	Yes	No				
Infection	VAP	HAP	Blood stream	Urinary track	Surgical wound	Meningitis
Site of *Acinetobacter* isolation	ETT	Sputum	Blood	Urine	Wound	CSF
Acinetobacter sensitivity	MDR	Non MDR				
Treatment	Polymyxin B	Ampicillin-sulbactam	Cefoperazone-sulbactam			
Date of *Acinetobacter* isolation						
Date of start of treatment						
No. of days						
Septic shock	Yes	No				
Microbiology	Yes	No				
CRP	Yes	No				
S/S	Yes	No				
30 days in patient mortality	Yes	No				

ESRF – defined as any individual requiring regular dialysis on a permanent basis.

Hepatitis B - defined as any person infected with hepatitis B virus evidenced by the presence of HBsAg (Hepatitis B Surface Antigen).

Hepatits C – defined as any person infected with hepatitis C virus with HCV antibody positive and HCV viral load detectable.

Liver cirrhosis- defined as any patient with ultrasound confirmation of liver cirrhosis.

Malignancy- malignant tumour affecting any system: hematological, gastro intestinal, thyroid, gynecological, pulmonary, hepatic, cerebral and osteoarticular.

Acinetobacter MDR - *Acinetobacter* Multi Drug Resistance – defined as isolate which is non-susceptible to at least one agent in three or more antibiotic classes.

No. of days – quantifies the number of days between detection of *Acinetobacter* infection and start of treatment.

Septic shock – sepsis with either lactate >2 mmol/L despite adequate fluid resuscitation or the patient is requiring vasopressors to maintain a mean arterial pressure of at least 65mmHg.

Microbiology – microbiological eradication of *Acinetobacter* at day 5 of treatment.

CRP – decrease of at least 50% of initial CRP level at day 5 of treatment.

S/S – resolution of signs and symptoms of patients at day 5 of treatment.

30 days in patient mortality – defined as any patient who died in hospital within 30 days period after starting treatment.

NEWS2 is the latest version of the National Early Warning Score (NEWS), first produced in 2012 and updated in December 2017, which advocates a system to standardize the assessment and response to acute illness.

The NEWS is based on a simple aggregate scoring system in which a score is allocated to physiological measurements, already recorded in routine practice, when patients present to, or are being monitored in hospital. Six simple physiological parameters form the basis of the scoring system:

- respiration rate
- oxygen saturation
- systolic blood pressure
- pulse rate
- level of consciousness or new confusion
- temperature

The NEWS (2) chart highlights that patients on supplemental oxygen score an additional 2 points, and holds a separate section for scoring O2 saturations in patients with chronic respiratory failure, in whom O2 saturation of 88-92% are recommended.

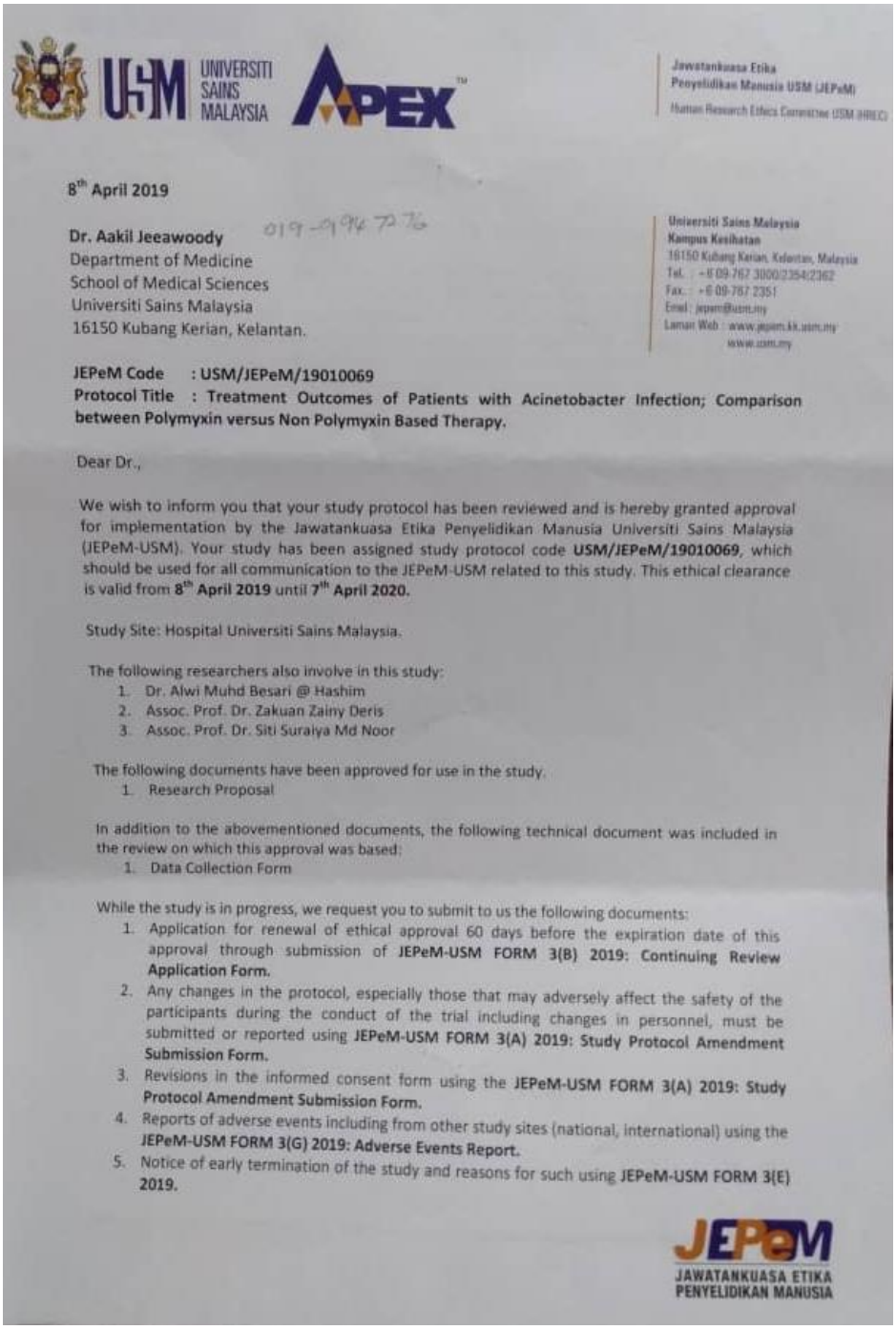
The NEWS2 score calculated on the day of initiation of either polymyxin or Ampicillin-sulbactam will be taken in this study

Table 10: NEWS2 Scoring System

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

(NEWS2 Standardising the assessment on acute illness severity in the NHS, Royal College of Physicians)

ETHICS APPROVAL LETTER



USM UNIVERSITI SAINS MALAYSIA

APEX

Jawatankuasa Etika
Penyelidikan Manusia USM (JEPeM)
Human Research Ethics Committee USM (HREC)

8th April 2019

Dr. Aakil Jeeawoody 019-0194726
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Email : jepem@usm.my
Laman Web : www.jepem.kk.usm.my
www.usm.my

JEPeM Code : USM/JEPeM/19010069
Protocol Title : Treatment Outcomes of Patients with Acinetobacter Infection; Comparison between Polymyxin versus Non Polymyxin Based Therapy.

Dear Dr.,

We wish to inform you that your study protocol has been reviewed and is hereby granted approval for implementation by the Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia (JEPeM-USM). Your study has been assigned study protocol code **USM/JEPeM/19010069**, which should be used for all communication to the JEPeM-USM related to this study. This ethical clearance is valid from **8th April 2019** until **7th April 2020.**

Study Site: Hospital Universiti Sains Malaysia.

The following researchers also involve in this study:
1. Dr. Alwi Muhd Besari @ Hashim
2. Assoc. Prof. Dr. Zakuan Zainy Deris
3. Assoc. Prof. Dr. Siti Suraiya Md Noor

The following documents have been approved for use in the study.
1. Research Proposal

In addition to the abovementioned documents, the following technical document was included in the review on which this approval was based:
1. Data Collection Form

While the study is in progress, we request you to submit to us the following documents:
1. Application for renewal of ethical approval 60 days before the expiration date of this approval through submission of **JEPeM-USM FORM 3(B) 2019: Continuing Review Application Form.**
2. Any changes in the protocol, especially those that may adversely affect the safety of the participants during the conduct of the trial including changes in personnel, must be submitted or reported using **JEPeM-USM FORM 3(A) 2019: Study Protocol Amendment Submission Form.**
3. Revisions in the informed consent form using the **JEPeM-USM FORM 3(A) 2019: Study Protocol Amendment Submission Form.**
4. Reports of adverse events including from other study sites (national, international) using the **JEPeM-USM FORM 3(G) 2019: Adverse Events Report.**
5. Notice of early termination of the study and reasons for such using **JEPeM-USM FORM 3(E) 2019.**

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JAWATANKUASA ETIKA PENYELIDIKAN MANUSIA

6. Any event which may have ethical significance.
7. Any information which is needed by the JEPeM-USM to do ongoing review.
8. Notice of time of completion of the study using **JEPeM-USM FORM 3(C) 2019: Final Report Form.**

Please note that forms may be downloaded from the JEPeM-USM website: **www.jepem.kk.usm.my**

Jawatankuasa Etika Penyelidikan (Manusia), JEPeM-USM is in compliance with the Declaration of Helsinki, International Conference on Harmonization (ICH) Guidelines, Good Clinical Practice (GCP) Standards, Council for International Organizations of Medical Sciences (CIOMS) Guidelines, World Health Organization (WHO) Standards and Operational Guidance for Ethics Review of Health-Related Research and Surveying and Evaluating Ethical Review Practices, EC/IRB Standard Operating Procedures (SOPs), and Local Regulations and Standards in Ethical Review.

Thank you.

"ENSURING A SUSTAINABLE TOMORROW"

Sincerely,

PROF. DR. HANS AMIN VAN ROSTENBERGHE
Chairperson
Jawatankuasa Etika Penyelidikan (Manusia) JEPeM
Universiti Sains Malaysia

PENERBIT USM
THE MALAYSIAN JOURNAL OF MedicalSciences
Website: http://www.mjms.usm.my Email: mjms.usm@gmail.com

GUIDELINES FOR AUTHORS

(*April 2011 Revision)*

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These guidelines are in accordance with the Uniform Requirement for Manuscripts Submitted to Biomedical Journals *(October 2008 revision)* of the International Committee of Medical Journal Editors.

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Authors are expected to provide detailed information about all relevant financial interests and relationships or financial conflicts within the past 5 years and for the foreseeable future, particularly those present at the time the research was conducted and through publication, as well as other financial interests (such as patent applications in preparation), that represent potential future financial gain. Authors may do so in the covering letter submitted via ScholarOne Manuscripts.

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All financial and material support for the research and the work should be clearly and completely identified in an Acknowledgment section of the manuscript. The specific role of the funding organization or sponsor in each of the following should be specified: design and conduct of the study; collection, management, analysis, and interpretation of the data; and

preparation, review, or approval of the manuscript.

Patient consent

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In experiments on human subjects, authors should mention whether the methods were in agreement with the ethical standards of the responsible committee (institutional and national) and the Declaration of Helsinki *(October 2008 revision)*. Similarly, the use of animals in research must conform to the institutional and national guidelines.

Types of Manuscripts

MJMS publishes the following types of manuscripts. Each type of manuscript has its own formats as outlined below:

- **Editorial (E)**: *Brief, substantiated commentary on subjects of topical interest.*

  Abstracts: Unstructured, not more than 150 words.

  Text: Not more than 1200 words (excluding references and figure/table legends).

  Tables and figures: Not more than 2.

  References: Not more than 20.

- **Original Article (OA)***: Report of original clinical or investigative laboratory research.*

  Abstract: Structured, not more than 275 words.

  The abstract is divided into Background, Methods, Results, and Conclusion.

  Text: Not more than 3500 words (excluding tables, figures, or references).

- **Review Article (RA)***: Overview of recent researches in a particular subject area suitable for a wide audience.*

  Abstract: Unstructured, not more than 275 words

  Text: Not more than 4500 words (excluding tables, figures, or references)

  References: Not more than 80

- **Case Report (CR)**: *Brief case report of unusual interest.*

  Abstract: Unstructured, not more than 175 words.

  Text: Not more than 2000 words (excluding tables, figures, or references)

  References: Not more than 10.

  Figures and tables: Not more than 3.

- **Brief Communications (BC)***: Description of a complete small investigation; or of new*

models, hypotheses, or innovative methods.

Abstract: Unstructured, not more than 175 words

Text: Not more than 1500 words (excluding tables, figures, or references)

Figures and tables: Not more than 3.

References: Not more than 20.

- **Special communications (SC)***: Article on an important issue in clinical medicine, public health, health policy, or biomedical research in a scholarly, thorough, well-referenced, systematic, or evidence-based manner.*

  Abstract: Unstructured, not more than 200 words.

  Text: Not more than 3000 words (excluding tables, figures, or references)

  References: Not more than 80.

- **Letter to the Editor (LE)***: Comments on articles published within 6 months in MJMS or articles of interest to the biomedical community.*

  Text: Not more than 500 words

  References: Not more than 6

  Submission: Email

- **Letters in reply (LR)***:Reply by authors*

  Text: Not more than 500 words

  References: Not more than 6

  Submission: Email

Preparation of Manuscript

General

- Text: Use subheadings for long articles and double-space all portions of the manuscript.
- Font: Times New Roman/Arial/Cambria, size 12pt, double-spaced, single column.
- Authors should number all of the pages of the manuscript consecutively, beginning with the title page, to facilitate the editorial process.
- Please note that, at the moment, we do not accept Microsoft Word 2007/2010 documents (*.docx). Please use Word's "Save As" option to save your document as (.doc) file type.

Each type of manuscript has its own formats; examples of published manuscript are available on our website. Authors may also consult the provided references—or other similar publications—for tips on preparing a scientific manuscript.

Manuscripts should be organized in the following order:

Title page

The title page should be sent as a **separate document** from the main text in ScholarOne Manuscripts. This document will not be available for reviewers as we employ a double-blind review process.

The title page should have the following information:

a. Article title without abbreviations

b. Running title/running head (a short title) of less than 50 characters

c. Authors' names and institutional affiliations: Full names are required; indicate last name with SMALL CAPS. For example, Mohammed Ali JAMALUDDIN.

d. Contact information for correspondence. The name, academic qualification, address,

telephone number, fax number, and email address of one of the authors who will be responsible for all communication concerning the manuscript are required.

e. Acknowledgements. Because the title page will not be sent to the reviewers, we recommend this section to be included in the title page.

Main document

Title

Abstract

The length of abstract depends on the type of manuscript submitted. The abstract should state the purpose of the study, a brief description of the procedures employed, main findings and principal conclusions. Abbreviations, footnotes, references, and subheadings should be avoided. For original articles, the abstract format is structured as Background, Methods, Results, and Conclusion. For other articles, the abstract format is unstructured.

Keywords

Authors must provide between 4 and 6 keywords that characterise the main topics of the article. Use recognised vocabularies related to the discipline discussed that are available in the MeSH thesaurus (http://www.nlm.nih.gov/mesh/2011/mesh_browser/MBrowser.html). We encourage the use of synonyms for terms provided in the article title; this is to aid database searches.

Text

The text of observational and experimental articles is usually (but not necessarily) divided into

Introduction, Materials (or Subjects) and Methods, Results, Discussion, and Conclusion. A case report is divided into Introduction, Case Report (or Series), and Discussion. Other types of manuscript may be divided into several sections, as seen necessary by the authors.

Long articles may need subheadings within some sections (especially Results and Discussion) to clarify their content. Subheadings representing different hierarchical levels must be readily distinguished by readers.

For example:

Heading 1	**Materials and Methods**	**Bold, title case**
Heading 2	*Enzymatic analyses*	*Italic, sentence case*
Heading 3	**Glutathione peroxidase assay**	**Bold, sentence case**
Normal text	xxxx xxx xxx xxx xxx	

List may be run into the text if the items are short, simple, and form a complete grammatical sentence, for example:

The lecturer will expound on (1) glyceraldehydes, (2) erythrose, (3) arabinose, and (4) allose.

Lists that contain several levels should be set vertically:

The animals were divided mainly into the following groups:

1. **Group 1:** Control (0.5 mL/kg saline, p.o.)
2. **Group 2:** Untreated diabetic (230 mg/kg NA and 65 mg/kg STZ)
3. **Group 3:** Diabetic + Combination-1 (1 mg/kg Pio + 50 mg/kg Met, p.o.)
4. **Group 4:** Diabetic + Combination-2 (1 mg/kg Pio + 0.2 mg/kg Gmp, p.o.)

5. **Group 5:** Diabetic + α-tocopherol (20 mg/kg, p.o.)
6. **Group 6:** Diabetic + insulin (1 IU/kg, s.c.)

Tables

Tables must be submitted **separately** from the main document. We **do not want** tables that are embedded in the text as an image. Tables must be numbered sequentially and in the order in which they are mentioned in the text. Tables must have brief descriptive title. Preferably, tables must be prepared according to the guides in *The Chicago Manual of Style* (15th edition), and are constructed in Microsoft Word.

Tips on constructing tables:

- Ensure that the table, including titles and footnotes, is complete enough to be understood without reference to the text.
- Make the table orderly, logical, and as simple as possible.
- For footnotes, use superscript lowercase letters, e.g., [a]Mean (SD), [b]Analysis of variance. Assign footnote letters in alphabetical order from left to right and from top to bottom.
- Expanded abbreviations are typically presented below the footnotes. Abbreviations defined in the text must be redefined as this practice allows the table to stand alone.

An example of table format suitable for MJMS is as depicted below:

Table 11: Association of CYP2D6 alleles and PANSS scores

	Subtotal Positive [a]		**Subtotal Negative [a]**		**Subtotal General [a]**		**Total PANSS [a]**	
*CYP2D6*1*	9.7	(3.52)	8.9	(3.86)	20.2	(4.46)	38.7	(10.11)
*CYP2D6*4*	9.8	(2.75)	7.3	(0.50)	22.3	(5.32)	39.3	(8.42)
*CYP2D6*5*	10.9	(2.78)	9.2	(3.74)	22.5	(6.26)	42.6	(11.13)
*CYP2D6*10*	9.4	(2.63)	8.8	(3.77)	20.6	(4.27)	38.9	(8.96)
Duplication	11.2	(5.01)	14.1	(7.67)	24.5	(8.76)	49.8	(19.31)
F statistic (*df*)	1.29	(4, 289)	4.44	(4, 289)	2.67	(4, 289)	3.22	(4, 289)
P value [b]	0.276		0.002		0.033		0.013	
NA	8.1	(2.19)	7.2	(0.65)	18.8	(2.90)	34.1	(4.86)
Total	9.6	(3.12)	8.9	(3.97)	20.5	(4.65)	39.1	(10.02)

[a]Mean (SD), [b]Analysis of variance (ANOVA). NA represents samples that were amplifiable during first PCR, but genotypes were not determined during the second PCR. Samples were screened for *CYP2D6*3, *4, *5, *6, *9, *10, *14, *17,* and duplication gene.

Source: Zahari et al. *Malaysian J Med Sci.* 2009;**16(3)**:13–21.

Figures

All figures must be uploaded **separately** from the main document in ScholarOne Manuscripts. Figures must be numbered sequentially and in the order in which they are mentioned in the text. Figure legends are needed for all figures.

We accept these figure types: Statistical graphs, charts, and simple diagrams.

Please submit graphs and charts in their original forms, e.g. the Excel/PowerPoint file. We **do not accept** graphs and charts **in JPEG/GIF.**

Photographic images (color photos, radiographs, ultrasound images, CT scans, MRI scans, electron micrographs, and photomicrographs).

Regardless of the application used, when your electronic artwork is finalised, please "save as" or convert the images to **one of the following formats** (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS: Vector drawings. Embed the font or save the text as "graphics".

TIFF: Colour or greyscale photographs (halftones): minimum of 300 dpi.

TIFF: Bitmapped line drawings: minimum of 1000 dpi.

TIFF: Combinations bitmapped line/half-tone (colour or greyscale): minimum of 500 dpi.

Please **do not**:

Supply embedded graphics in your word processor (spreadsheet, presentation) document.

Supply files that are optimised for screen use (like GIF, BMP, PICT, WPG); the resolution is too low.

Supply files that are too low in resolution; 72 dpi web-quality graphics in which

colours are not realistic, text is illegible, or images are pixelated.

Submit graphics that are disproportionately large for the content.

Combine figure legends and figure in a textbox or frame.

Videos

From 2011, we welcome videos in manuscripts. Videos may be useful for demonstrating complex laboratory, surgical or medical procedures. The demonstration of the experiment must be shown in orderly fashion, including a demonstration of equipment and reagent. Researchers should be properly attired when handling animals, reagents, and chemicals.

Preferred settings of videos:

- Audio codec: AAC
- Sample audio bit rate: 128 kbit/s
- Video codec: H.264
- Video resolution: 480 vertical lines or better
- Maximum file size: 30 MB
- Format: mov, avi, mpg, mpeg, mp4, mkv, flv, wmv

The video should make a specific point; particularly, it should demonstrate the features described in the text of the manuscript. Special effects or texts are not permitted to be inserted in the video. Authors who intend to submit videos must have the necessary expertise in video post-production.

References

Citation in text

References should be numbered consecutively in the order in which they are first mentioned in the text **(citation-sequence)** —the Vancouver style. Identify references in text, tables, and legends by Arabic numerals in parentheses, for example: (2), (3–5).

List of references

For formatting end references, we recommend following the guidelines of the **Council of Science Editors (CSE)**, which can be accessed through http://library.duke.edu/research/citing/workscited/.

Author is also requested to provide the **digital object identifier (DOI)** for each DOI-assigned citation. DOI is usually available in the bibliographic information and can be retrieved from CrossRef (http://www.crossref.org/) by using **free DOI lookup** (http://www.crossref.org/guestquery/) or **simple text queries** (http://www.crossref.org/SimpleTextQuery/). Please note that failure to comply with this direction may result in a delay in the manuscript publication.

Journal article

Author(s) surname(s) Initial(s). Title of article. *Journal title**. Year of publication;**Volume(Issue)**:Pagination. DOI.

*A journal title should be abbreviated according to the style used in PubMed or ISI.

Johnson LA, Jackson DG. Inflammation-induced secretion of CCL21 in lymphatic endothelium is a key regulator of integrin-mediated dendritic cell transmigration. *Int Immunol*. 2010;**22(10)**:839–826. doi: 10.1093/intimm/dxq432.

If there are more than **6** authors, list the first 6 authors and use “et al.” for the subsequent

authors.

Asp J, Steel D, Jonsson M, Ameen C, Dahlenborg K, Jeppsson A, et al. Cardiomyocyte clusters derived from human embryonic stem cells share similarities with human heart tissue. *J Mol Cell Biol*. 2010;**2(5)**:276–238. doi: 10.1093/jmcb/mjq022.

If a journal article is forthcoming, conclude the references with the estimated date of publication.

Hassan R, Aziz AA. Computed tomography imaging of injuries from blunt abdominal trauma: A pictorial essay. *Malaysian J Med Sci*. Forthcoming 2010 Jun.

Sometimes, instead of a particular volume or issue, a supplement or other parts such as special issue may be published instead. Place such supplements and parts after the year of publication.

Al-Tawfiq JA, Clark TA, Memish ZA. Meningococcal disease: The organism, clinical presentation, and worldwide epidemiology. *J Travel Med*. 2010;**17 Suppl**:S3–S8. doi: 10.1111/j.1708-8305.2010.00448.x.

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Carlson BM. *Human embryology and developmental biology*. 3rd ed. St Louis: Mosby; 2004.

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Anderson RJ, Schrier RW. Acute renal failure. In: Braunwald E, Isselbacher KJ, Petersdorf RD, editors. *Harrison's principles of internal medicine*. 15th ed. New York: McGraw-Hill; 2001. p. 1149–1155.

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Rabbani SI, Devi K, Khanam S. Role of pioglitazone with metformin or glimepiride on oxidative stress-induced nuclear damage and reproductive toxicity in diabetic rats. *Malaysian J Med Sci* [Internet]. 2010 [cited 2010 Mar 21]**;17(1)**:3–11. Available from: http://ernd.usm.my/journal/journal/02-171OA1pioglitazone.pdf.

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Author(s) surname(s) Initial(s). Title of article [Internet]. Place of publication: Publisher; Year of publication [cited YYYY MMM DD]. Available from: URL.

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RAW DATA

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LIST OF ABBREVIATIONS

CFU : Colony forming unit

CRAB : Carbapenem resistant *Acinetobacter baumannii*

CRP : C reactive protein

CSF : Cerebrospinal fluid

ESRF : End stage renal failure

HAP : Hospital acquired pneumonia

HDU : High dependency unit

Hep B : Hepatitis B

Hep C : Hepatitis C

HUSM : Hospital Universiti Sains Malaysia

ICU : Intensive care unit

MDR : Multi drug resistant

MDRAI : Multi drug resistant *Acinetobacter* infection

NEWS2 : National Early Warning Score 2

UKJEH : Unit Kawalan Jangkitan & Epidemiologi Hospital

USM : Universiti Sains Malaysia

VAP : Ventilator associated pneumonia

LIST OF SYMBOLS

%	percent
=	equal to
>	more than
<	less than
≤	less than or equal to
≥	more than or equal to
℃	degrees Celsius
ml	millilitre
vs.	versus
SpO2	saturation in oxygen
CFU/ml	Colony forming unit per millilitre
cells/mm^3	cells per millimetre cube

ABSTRAK

Latarbelakang: Peningkatan ketahanan *Acinetobacter* terhadap hampir kesemua antibiotik yang berada di pasaran merupakan suatu kebimbangan utama. Pada masa ini, terdapat pilihan pengubatan yang terhad.

Objektif: Tujuan utama kajian ini adalah untuk membandingkan keberkesanan amalan sulbactam terhadap polymyxin B dalam rawatan jangkitan *Acinetobacter*.

Kaedah: Ini merupakan kajian retrospektif rekod kes dalam jangkamasa setahun (1 Januari 2018 hingga 31 Disember 2018) di Hospital Universiti Sains Malaysia. Kajian ini melibatkan pesakit yang berumur sekurang-kurangnya 18 tahun, dan mempunyai bukti klinikal dan mikrobiologikal jangkitan *Acinetobacter*.

Keputusan: 34 pesakit menerima polimiksin dan 38 telah menerima sama ada ampicillin-sulbactam atau cefoperazone-sulbactam. 24 (63.2%) daripada kumpulan bukan polymyxin mencapai kejayaan klinikal manakala 12 (38.2%) mencapai kejayaan klinikal dalam kumpulan polymyxin. 26 pesakit (68.4%) yang dirawat dengan bukan polymyxin mencapai kejayaan mikrobiologikal berbanding dengan 18 (52.9%) yang dirawat dengan polymyxin. Kematian adalah rendah dalam kumpulan bukan polymyxin dengan jumlah 17 sahaja (44.7%) berbanding dengan 23 kematian (67.6%) dalam kumpulan polymyxin. Regresi logistik pelbagai menunjukkan bahawa kegagalan mikrobiologikal terkait secara signifikan dengan 30 hari kematian pesakit.

Kesimpulan: Penemuan terpenting kajian kami adalah sulbactam yang sebenarnya lebih berkesan daripada polymyxin dalam merawat jangkitan *Acinetobacter*.

Kata kunci: *Acinetobacter*, polymyxin, sulbactam, berkesan, kematian

ABSTRACT

Background: The growing resistance of *Acinetobacter* to almost all commercially available antibiotics is of major concern. Limited therapeutic options are currently available.

Objectives: The aim of the study was to compare the efficacy of sulbactam regime to that of polymyxin B in the treatment *Acinetobacter* infection.

Methods: This was a retrospective study of case records over one year period (1st January 2018 to 31st December 2018) at the Hospital Universiti Sains Malaysia. Patients of least 18 years old, with clinical and microbiological evidence of *Acinetobacter* infection, were enrolled in the study.

Results: 34 patients received polymyxin and 38 received either ampicillin-sulbactam or cefoperazone-sulbactam. 24 (63.2%) from the nonpolymyxin group achieved clinical success while 13 (38.2%) achieved clinical success in the polymyxin group. 26 patients (68.4%) treated with nonpolymyxin achieved microbiological success compared to 18 (52.9%) treated with polymyxin. Mortality was lower in the nonpolymyxin group with 17 deaths (44.7%) compared to 23 deaths (67.6%) in the polymyxin group. Multiple logistic regression showed that microbiological failure was significantly associated with 30 days in patient mortality.

Conclusion: The most important finding of our study is that sulbactam appears to have a better efficacy compared to polymyxin in treating *Acinetobacter* infection.

Keywords: *Acinetobacter*, polymyxin, sulbactam, efficacy, mortality

CHAPTER 1

INTRODUCTION

Acinetobacter is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria. It comprises of more than 50 species, most of which are nonpathogenic environmental organisms. The most common infection-causing species is *Acinetobacter baumannii,* followed by *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Acinetobacter baumannii* has the potential of spreading among hospitalized patients by virtue of its ability for exogenous colonization of human body (throat, gastrointestinal tract, skin) and its high tolerance of difficult conditions (survivability in the environment up to 1 month) (Wendt et al. 1997).

The ability of *Acinetobacter* to accumulate diverse mechanisms of resistance, has led to the emergence of strains that are resistant to all commercially available antibiotics (Lolans et al., 2006). *Acinetobacter baumannii* forms part of the ESCAPE organisms, which are predominantly health care-associated organisms that have the potential for substantial antimicrobial resistance (De Rosa et al. 2015, Rice et al. 2008).

In the year 2011, the European and United States Centres for Disease Control and Prevention (ECDC and CDC) joined to propose specific definitions for characterizing drug resistance in organisms that cause many health care-associated infections (Magiorakos et al. 2012). The following definitions were established based on the extent of resistance to antibiotics that would otherwise serve as treatments for *Acinetobacter* (cephalosporins, fluoroquinolones and carbapenems)

- Multidrug-resistant: isolate is non-susceptible to at least one agent in three or more antibiotic classes
- Extensively drug-resistant: isolate is non-susceptible to at least one agent in all but two or fewer antibiotic classes

- Pandrug-resistant: isolate is non-susceptible to all agents

As from the 1980s, the resistant strains of *Acinetobacter* became more and more common causes of nosocomial infections globally (Gaynes et al. 2005, Rhomberg et al. 2007, Tatman-Otkun et al. 2004). Based on a 2009 report of surveillance data from more than 100 centers worldwide (Meropenem Yearly Susceptibility Test Information Collection; MYSTIC), 61 percent of *Acinetobacter* isolates were resistant to ceftazidime and 67 percent were resistant to ciprofloxacin (Rhomberg et al. 2009). Emergent carbapenem-resistant strains have been demonstrated by other worldwide studies with high rates of carbapenem resistance in some locations (Giske et al. 2008, Jean et al. 2011, Manikal et al. 2003, Peleg et al. 2006, Playford et al. 2007). For instance, the prevalence of carbapenem-resistant *Acinetobacter baumannii* at two teaching hospitals in the UK increased from 47 to 77 percent from 2010 to 2012 (Freeman et al. 2015) while in one referral hospital in northern Vietnam, more than 90 percent of isolates were carbapenem resistant (Van et al. 2014). The reported prevalence of carbapenem resistance among *Acinetobacter baumannii* isolates is also quite high in the countries of the Arab League, ranging from 36 to 100 percent (Moghnieh et al. 2018). The epidemiology of serious hospital-acquired infections has been influenced by the rising prevalence of antimicrobial resistance among *Acinetobacter baumannii* isolates. One systematic review showed that carbapenem-resistant and multidrug-resistant *Acinetobacter baumannii* accounted for 65 and 59 percent, respectively, of all hospital-acquired infections among intensive care unit patients in Southeast Asia (Teerawattanapong et al. 2018).

Polymyxin B and polymyxin E (Colistin) are the most commonly used agents for *Acinetobacter* isolates resistant to first-line agents. There are no randomized trials addressing their efficacy, largely because they are reserved for use in the setting of highly resistant organisms. Colistin had some success for the treatment of *Acinetobacter* pneumonia, bacteraemia, and meningitis (Garnacho-Montero et al. 2003, Levin et al. 1999). Among nine studies (178 patients) that did

not include a comparator treatment, the pooled clinical response rate for intravenous colistin was 66%. However, one small series of 20 cases of nosocomial pneumonia that was not included in the analysis reported a success rate of only 25 percent (Levin et al. 1999). Nephrotoxicity is the most notorious adverse effect associated with systemic colistin and has been reported in up to 36 percent of patients (Falagas et al. 2006). Neurotoxicity is another important side effect but consists mainly of paraesthesia and is relatively uncommon. Colistin dosing depends on the available preparation and should be adjusted in patients with impaired renal function. Polymyxin B is associated with lower rates of nephrotoxicity than Colistin.

Sulbactam, a beta lactamase inhibitor, has shown to have good in vitro activity against *Acinetobacter* species (Urban et al. 1993). In HUSM, sulbactam is available in combination form namely as ampicillin-sulbactam and cefoperazone-sulbactam. Several studies have suggested that sulbactam might be effective in *Acinetobacter* infection. For example, high dose ampicillin-sulbactam was evaluated as an alternative treatment of late onset ventilator associated pneumonia from multidrug resistant *Acinetobacter baumannii* (Betrosian et al. 2007). The aim of the study was to evaluate the efficacy and safety of two high dose treatment regiments of ampicillin-sulbactam for multi-drug resistant *Acinetobacter baumannii* VAP. It was a randomised prospective trial in Hippokration General Hospital in Athens consisted of 27 patients. Mortality rates did not differ significantly between the two groups. No major adverse reactions were recorded. The conclusion that the study supported the use of high dose regimen of ampicillin-sulbactam for MDR *Acinetobacter baumannii* VAP. However due to the small sample size, the result of the study was not statistically strong.

A retrospective case series study in Korea evaluated the efficacy of high dose sulbactam treatment for ventilator associated pneumonia caused by carbapenem resistant *Acinetobacter baumannii* (Jeong et al. 2016). The conclusion of the study was that high dose sulbactam could be effective for the treatment of CRAB ventilator associated pneumonia. However early clinical failure was

common and is associated with a higher mortality with the treatment. The sample size was small and the study was not a randomised clinical trial.

In 2013, a systematic review and meta-analysis of sulbactam based therapy for *Acinetobacter baumannii* infection was published (Chu et al. 2013). This meta-analysis consisted of four studies three of which were retrospective while one was prospective. Treatment with sulbactam was compared to treatment with other classes of antibiotics. The results suggested that sulbactam-based therapy may be efficacious to alternative antimicrobial therapy for the treatment of *Acinetobacter* infection. However, only a very small number of trials were included and none of the trial were randomised trials. Furthermore the number of participants in the studies was relatively small and thus the power of the study was not strong enough.

Another study compared the efficacy of ampicillin/sulbactam and Colistin in the treatment of multidrug resistant *Acinetobacter baumannii* ventilator associated pneumonia (Betrosian et al. 2008). This was a prospective cohort study in 28 adults in the intensive care units in Hippokration General Hospital in Athens. The conclusion was that Colistin and high dose ampicillin/sulbactam were comparably safe and effective treatments for critically ill patients with MDR *Acinetobacter baumannii* VAP. However, the sample size of this study was small and the statistical power of this study was weak.

In addition, one retrospective study compared ampicillin/sulbactam with polymyxin for the treatment of infections caused by carbapenem- resistant *Acinetobacter* species (Oliveira et al. 2008). The study consisted of a total of 190 patients and was carried out in 2 large teaching hospitals in Brazil. The findings of the study was that ampicillin/sulbactam appeared to be more efficacious than polymyxin, which was an independent factor associated with mortality during treatment. However, the polymyxin group consisted of significantly older patients, more frequently submitted to surgical procedures and had more patients with cancer.

Furthermore, a 2003 retrospective study consisted of treating 40 MDR *Acinetobacter baumannii* infected patients with intravenous ampicillin/sulbactam (Levin et al. 2003). The median dose of ampicillin/sulbactam was 6g/3g. There were no observed adverse effects and that study indicated that ampicillin/sulbactam might be a good and safe therapeutic option to treat severe *Acinetobacter baumannii* nosocomial infections. However the study was not a randomised clinical trial.

In 1998, a prospective study was published whereby sulbactam was evaluated in 40 patients with non-life threatening multiresistant *Acinetobacter baumannii* infection in the Hospital de Bellvitge in Barcelona (Corbella et al, 1998). 18 patients received intravenous sulbactam alone versus 24 who received intravenous ampicillin-sulbactam. The results of the study suggested that sulbactam might prove effective for non-life threatening *Acinetobacter baumannii* infections. However, its role in the treatment of severe infections was unknown.

These studies have showed promising results of sulbactam based therapy in *Acinetobacter* infection. However, to our knowledge, no similar study was carried out in Malaysia before. We wanted to assess the outcomes of treating *Acinetobacter* infection in our population with sulbactam. The hypothesis was that sulbactam was as effective as polymyxin B in treating *Acinetobacter* infection. Thus, this study's results would provide a better insight on the accuracy of the hypothesis.

CHAPTER 2

OBJECTIVES OF THE STUDY

GENERAL OBJECTIVE

- To study the outcomes of patients with *Acinetobacter* infection.

SPECIFIC OBJECTIVES

1. To determine the proportion of patients with *Acinetobacter* infection treated with polymyxin versus non polymyxin based treatment.

2. To determine the association between polymyxin and non polymyxin based therapy among patients with *Acinetobacter* infection in terms of health outcomes: success versus failure.

CHAPTER 3

MANUSCRIPT

TITLE

Treatment outcomes of patients with *Acinetobacter* infection; comparison between polymyxin versus non polymyxin based therapy

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ABSTRACT

Background: The growing resistance of *Acinetobacter* to almost all commercially available antibiotics is of major concern. Limited therapeutic options are currently available.

Objectives: The aim of the study was to compare the efficacy of sulbactam regime to that of polymyxin B in the treatment *Acinetobacter* infection.

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Conclusion: The most important finding of our study is that sulbactam appears to have a better efficacy compared to polymyxin in treating *Acinetobacter* infection.

Keywords: *Acinetobacter*, polymyxin, sulbactam, efficacy, mortality

INTRODUCTION

Acinetobacter species is a recognised pathogen implicated in a wide range of nosocomial infections. Its growing resistance to almost all commercially available antibiotics is of major concern. Till date, there has a lack of randomised clinical trials to evaluate the best antimicrobial regimen for treating *Acinetobacter* infections. In clinical practice, Polymyxin B and Colistin (Polymyxin E) are being used. They have good in vitro activity against many gram negative bacilli including *Acinetobacter* species. The major adverse effects are nephrotoxicity, neurotoxicity and neuromuscular blockade (Evans et al. 1999, Horton et al. 1982). At the Hospital Universiti Sains Malaysia, Polymyxin B is the current available therapy for the *Acinetobacter* infection. It is a relatively expensive treatment and therefore its use is strictly regulated. Sulbactam, a beta lactamase inhibitor, has shown to have good in vitro activity against *Acinetobacter* species (Urban et al, 1993). Some studies have suggested that sulbactam might be effective in *Acinetobacter* infection (Betrosian et al. 2007, Betrosian et al. 2008, Chu et al. 2013, Corbella et al. 1998, Jeong et al. 2016, Levin et al. 2003, Oliveira et al. 2008). At our centre, sulbactam is available in combination forms namely as ampicillin-sulbactam and cefoperazone-sulbactam. Unasyn® is sulbactam combined with ampicillin in a fixed 2:1 ratio while sulperazone® is sulbactam combined with cefoperazone in a ratio of 1:1. Sulbactam is a well-tolerated drug with the main adverse effects being pain at the site of injection, diarrhoea and rash. In addition, the cost of the treatment with sulbactam is affordable to the general public. The aim of the study was to compare the efficacy of sulbactam regime to polymyxin B in the treatment *Acinetobacter* infection.

METHODOLOGY

Study population

This was a retrospective study of case records over one year period (1st January 2018 to 31st December 2018) at the Hospital Universiti Sains Malaysia (HUSM). HUSM is a tertiary care teaching hospital located in the north east state of Kelantan in Malaysia. The enrolled cases were hospitalised patients who were at least 18 years old with clinical evidence of infection and with isolation of *Acinetobacter* species from a specific culture site. Those patients who were already on treatment with either polymyxin B or sulbactam for other concomitant infection, on the day of isolation of *Acinetobacter*, were excluded. The demographic, clinical and laboratory data from the patient's file were collected. The study cohort was divided into two groups namely the polymyxin group and the nonpolymyxin group. Each infection was defined using some specific criteria as mentioned below.

For instance, pneumonia was defined as patient having a new or progressive radiographic parenchymal lung infiltrate with some signs that the infiltrate was infectious in origin. This required the presence of at least 2 of the following signs: temperature alteration (less than 36°C or at least 38.3°C), a white blood cell count less than 5000 cells/mm^3 or more than 10,000 cells/mm^3, or purulent-appearing sputum or endotracheal aspirate. Hospital Acquired Pneumonia (HAP) referred to the development of parenchymal lung infection after at least 48 hours of hospitalisation. On the other hand, if the infection developed after the patient underwent intubation and received mechanical ventilation for at least 48 hours, the condition was termed Ventilator Associated Pneumonia (VAP).

Bloodstream Infection included the primary, secondary and central line associated bloodstream infections.

- Primary bloodstream infection was defined as a laboratory confirmed bloodstream infection that was not secondary to an infection at another body site.
- Secondary bloodstream infection was defined as a bloodstream infection that was thought to be seeded from a site-specific infection at another body site.
- Central line-associated bloodstream infection was defined as a laboratory confirmed bloodstream infection where an eligible bloodstream infection organism was identified and an eligible central line was present on the laboratory confirmed bloodstream infection day of event or the day before.

Surgical site infection occurred within 30 days of surgery and involved any part of the body deeper than the fascia/muscle layers that was opened or manipulated during the operative procedure. The patient had at least one of the following:

- purulent drainage from a drain that is placed into the organ/space
- organism(s) identified from fluid or tissue in the organ/space by a culture
- an abscess or other evidence of infection involving the organ/space that is detected on gross anatomical or histopathologic exam, or imaging test evidence suggestive of infection.

Urinary tract infection was defined as patient having at least one of the following signs or symptoms: fever (temperature of at least 38.0°C), suprapubic tenderness, costovertebral angle pain or tenderness, urinary urgency, urinary frequency or dysuria. In addition, the patient's voided urine should yield a culture of at least 10^5 CFU/ml of not more than 2 species of microorganisms.

Meningitis was defined as patient having at least two of the following: fever (temperature of at least 38.0°C) or meningeal sign(s), cranial nerve sign(s) with

- Organism identified from cerebrospinal fluid (CSF) by a culture
- organism seen on Gram stain of CSF
- increased white cells, elevated protein, and decreased glucose in CSF (per reporting laboratory's reference range)

National Early Warning Score 2 (NEWS2) is a scoring system used for the assessment and response to acute illness. Six parameters form the basis of the scoring system: respiratory rate, oxygen saturation, systolic blood pressure, heart rate, level of consciousness and temperature. The NEWS2 holds a separate section for scoring oxygen saturations in patients with chronic respiratory failure, in whom oxygen saturation of 88-92% are recommended. The NEWS2 score calculated on the day of initiation of polymyxin, ampicillin-sulbactam and cefoperazone-sulbactam was taken into account in this study.

Table 1: NEWS2 scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

(NEWS2 Standardising the assessment on acute illness severity in the NHS, Royal College of Physicians)

LOW score: an aggregate NEWS2 score of 1–4

MEDIUM score: an aggregate NEWS2 score of 5 or 6.

HIGH score: an aggregate NEWS2 score of 7 or more.

Definition of Outcome Events

The treatment efficacy was assessed on day 5 of treatment. It comprised of 3 outcomes: microbiological response, clinical response and 30 days in patient mortality.

The clinical response was defined as

- Success if signs and symptoms improved and/or a decrease of at least 50% on initial CRP at day 5 of treatment.
- Failure if symptoms and signs persisted or worsened at day 5 of treatment.

The microbiological response was defined as

- Success if there was eradication of *Acinetobacter* species from culture at day 5 of treatment.
- Failure if persistence of *Acinetobacter* species at day 5 of treatment.

30 days in patient mortality was defined as any death of *Acinetobacter* infected patients within 30 days of starting treatment in hospital setting.

Statistical Analysis

Data was entered and analysed using SPSS version 24. The results were expressed in terms of numbers and percentages or mean and standard deviation. The categorical variables were tested using the chi square test while the student's t-test was used for continuous variables. A p-value of <0.05 was considered significant. In addition, logistic regression analysis was carried out to evaluate the potential independent risk factors for mortality.

Ethical Issue

This study was conducted in accordance with the principles laid by the 18th World Medical Assembly (Helsinky, 1964), and all subsequent amendments. It was approved by the Human Research Ethics Committee of USM (JEPeM) on the 8th April 2019 (Reference number: **USM/JEPeM/ 19010069**). The official authorisation to access patients' folders was granted from the Director of HUSM. The Infectious Control and Epidemiology Unit (UKJEH) of HUSM was contacted in order to get the list of patients with culture positive for *Acinetobacter* for the intended time period. The patients' personal identification and clinical data were confidential. No conflict of interest was involved in this study and no payment was given or received from any company or organization. All of the information obtained from the medical records was recorded in a password-protected computer folder to prevent any intentional or unintentional breach of patient's confidentiality.

RESULTS

A total of two hundred and eighteen cases were reviewed. Among these cases, one hundred and forty were either contaminants or colonisers. Only seventy-eight were *Acinetobacter* infections. Six of them were excluded as they were treated with a different antibiotic (piperacillin-tazobactam). Purposive sampling was carried out. Thirty-four received polymyxin treatment, twenty-four received ampicillin-sulbactam and fourteen received cefoperazone-sulbactam (Table 2). Thus, the nonpolymyxin group had a total of thirty-eight patients (52.8%).

The initial sample size calculated was one hundred and forty. However, at the end of the study, only seventy-two cases were obtained. The exact prevalence of acinetobacter infection in HUSM was unknown, so it was difficult to determine the proportion of *Acinetobacter* infection beforehand. As this was a retrospective study and we were limited in time, we could not afford to search for more cases in order to meet the calculated sample size. Furthermore, there were twenty case notes which could not be traced during the study period. .

The characteristics of the study population are summarised in the Table 3. There were forty-six (63.9%) males and the mean age was 55.0 years old. Forty patients (55.6%) were admitted to ICU while fifteen (20.8%) were admitted in HDU and seventeen (23.6%) were admitted to general wards. Four (5.6%) had end stage renal disease while three (3.4%) had chronic liver disease. Thirty-one (43.1%) were diabetics while eleven (15.3%) had a specific underlying malignant condition. The mean NEWS2 Score of the population was 6.8. Sixty-six (91.7%) were infected with multidrug resistant *Acinetobacter* species.

The majority of the *Acinetobacter* infections was ventilator associated pneumonia, with twenty-four (70.6%) in the polymyxin group versus twenty-one (55.3%) in the nonpolymyxin group (Table 4). Five (14.7%) and nine (23.7%) in the polymyxin and nonpolymyxin group respectively had bloodstream infection. There was only one case (2.9%) of meningitis treated with polymyxin

while on the other hand there was only one case (2.6%) of urinary tract infection treated in the nonpolymyxin group. Two (5.9%) hospital acquired pneumonia were in the polymyxin group while three (7.9%) hospital acquired pneumonia cases were in the nonpolymyxin group.

In the polymyxin group, the mean age was 50.6 years old compared to 58.9 years old in the nonpolymyxin group (Table 5). The mean NEWS2 score of the polymyxin group was higher compared to that of the nonpolymyxin group (8.1 vs. 5.6). Seventeen (50%) in the polymyxin group had septic shock compared to three (7.9%) in the nonpolymyxin group. Thirty-three cases (97.1%) of multidrug resistant acinetobacter infection were present in the polymyxin group compared to thirty-three (86.8%) in the other group. There were more diabetics with twenty (52.6%) in the nonpolymyxin group versus eleven (32.4%) in the polymyxin group. Two patients (5.9%) had end stage renal disease in the polymyxin group and there were two patients (5.3%) in the nonpolymyxin group as well. Chronic liver disease was present in two patients (5.9%) in the polymyxin group and one patient (2.6%) in the nonpolymyxin group. Six (17.6%) had a specific underlying malignant condition in the polymyxin group and five (13.2%) in the nonpolymyxin group. Twenty-three (67.6%) were males in the polymyxin group and similarly there were twenty-three (60.5%) males in the nonpolymyxin group. Twenty-four (70.6%) in the polymyxin group required ICU admission compared to sixteen (42.1%) in the nonpolymyxin group. The mean number of days between isolation of *Acinetobacter* and start of treatment in both group is almost similar: 1.79 days in the polymyxin group vs. 1.42 days in the nonpolymyxin group.

Twenty-four (63.2%) from the nonpolymyxin group achieved clinical success while in the polymyxin group only thirteen (38.2%) achieved clinical success (Table 6). Twenty-six (68.4%) achieved microbiological success in the nonpolymyxin group versus eighteen (52.9%) in the polymyxin group. Mortality was lower in the nonpolymyxin group with seventeen deaths (44.7%) compared to twenty-three deaths (67.6%) in the polymyxin group.

The logistic regression analysis results for the 30-day in patient mortality is shown in Table 7. Based on p-value <0.25, the following variables were selected to multiple logistic regression analysis: NEWS2 score, male gender, malignancy, septic shock, polymyxin group, and microbiological outcome.

By using method Forward LR for variable selection, variable microbiological outcome remained in the model for analysis multiple logistic regression (Table 8). Thus, microbiological failure was significantly associated with the 30-days in patient mortality.

DISCUSSION

Acinetobacter is known to be one of the most frequent infective organisms in intensive care units. One study showed that 54.9% of *Acinetobacter* species isolates were obtained from ICUs, 36.7% and 8.4% from the medical and surgical units respectively (Uwingabiye et al. 2016). Another study noted that *Acinetobacter baumannii* was more frequently associated with infection among patients in the ICU (63.9%) compared to patients admitted to medical (52.8%) and to surgical wards (52.9%) (Villar et al. 2014). Similarly, our study found a predominance of *Acinetobacter* infections in intensive care unit. Forty patients (55.6%) were from ICU while fifteen (20.8%) were from HDU and seventeen (23.6%) were from general wards.

The majority of the *Acinetobacter* infections was ventilator associated pneumonia, with twenty-four patients (70.6%) in the polymyxin group versus twenty-one (55.3%) in the nonpolymyxin group. Five (14.7%) and nine (23.7%) in the polymyxin and nonpolymyxin group respectively had bloodstream infection. Our study was in concordance with other studies whereby VAP was proved to be the most common *Acinetobacter* infection. For instance, one study showed that VAP accounted for 73.8% of "*Acinetobacter baumannii*" infection (Duszynska et al. 2018) while

another study concluded that pneumonia was the most common site of "*Acinetobacter baumannii*" infection (53.1%) (Castilho et al. 2017).

There was one case (2.9%) of multidrug resistant *Acinetobacter* meningitis in our study which was detected in the CSF of a 22-year-old patient who underwent neurosurgical intervention for pineal gland tumour. The patient was treated with polymyxin but unfortunately, the treatment was unsuccessful and the patient passed away in ICU. This case outlines the difficulty in treating *Acinetobacter* meningitis and highlights its associated high mortality rate. Chen et al. (2005) noted a 30% mortality rate among patients with *Acinetobacter* meningitis while Rodriguez et al. (2008) noted a mortality rate of 33.3% in patients with nosocomial neurosurgical meningitis.

It has been a common practice at our hospital to use polymyxin for the younger and more severely ill patient infected with *Acinetobacter* in order to maximise their prospect of cure and survival. This was evidenced by our data results that showed a lower mean age in the polymyxin group (58.9 years vs. 50.6 years) but with a higher percentage of septic shock (50% vs. 7.9%).

43.1% of the study population were diabetics. Even though there were more diabetics in the nonpolymyxin group than in the polymyxin group (52.6% vs. 32.4%), our study did not show any relationship between diabetes and the outcomes in the two groups. Furthermore, diabetes did not have any significant impact on the mortality. This is in contrast to the study led by Leung et al. (2019) which found that mortality was higher in diabetic patient with *Acinetobacter* infection.

In terms of outcomes, the nonpolymyxin group fared better compared to the polymyxin group. Twenty-four patients (63.2%) from nonpolymyxin group achieved clinical success while in the polymyxin group only thirteen (38.2%) achieved clinical success. This success achieved statistical significance ($p=0.035$). Levin et al. (2003) studied twelve patients with ampicillin-sulbactam and the results showed 67.5% had clinical improvement. Corbella and al. (1998) treated forty-two cases of non-life threatening *Acinetobacter* infection with sulbactam and noted a clinical