POLYMYXIN B THERAPY FOR MULTIDRUG RESISTANT GRAM NEGATIVE INFECTIONS : OUTCOME AND RISK FACTORS FOR TREATMENT FAILURE IN CRITICAL CARE

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF MEDICINE (ANAESTHESIOLOGY)

UNIVERSITI SAINS MALAYSIA

MAY 2015

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious, Most Merciful. Praise to Allah, by His Grace, this manuscript was able to be completed.

This manuscript appears in its current form due to the assistance and guidance of several people. I would therefore like to offer my sincere thanks to all of them.

My sincere thanks to my supervisor, Associate Prof. Dr. Mahamarowi Omar for his valuable guidance and advice in the preparation of this dissertation.

Special thanks to Associate Prof. Dr. Saedah Ali as my co-supervisor, and Associate Prof. Dr. Zakuan Zainy Deris as my microbiology supervisor as well as Professor Dr Shamsul Kamaruljan, our Head of Department of Anaesthesiology and Intensive Care HUSM, for their advices and encouragement.

I warmly thank Tn. Hj Zainol Abidin Hamid and Pn. Khairul Bariyah from Pharmacy Dept, my ICU staffs; Sr Rokiah Ismail, JR Norhamilah Hassan, JR Zulafiza Ali, JR Nor Asmah Ismail, UKJEH staffs; Sr Narizan Mohd Idris, Jr Azrahamiza Abdullah, Jr Nor Reah Mustafa, and Jr Silamai A/P Ea Chum, and also all Record Unit staffs for their support and assistance.

To my dearest husband Mr Roslan Mamat, my beautiful mother Mrs Hjh Aisyah, my adorable sons Ajmal and Ajwad, and all family members, thanks for their prayer and of course, the endless love, support and patience.

May Allah (SWT) bless all of us.

AMIN.

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ABBREVIATIONS

MDROs	Multi-drug Resistant Organisms
MDR	Multi-drug Resistant
MDRGN	Multi-drug Resistant Gram Negative
VAP	Ventilator-associated Pneumonia
BSI	Blood Stream Infection
CRE	Carbapenem Resistant Enterobacteriaciae
UTI	Urinary Tract Infection
SSI	Surgical Site Infection
MIC	Minimum Inhibitory Concentration
ESBL	Extended Spectrum B-lactamase
KPC	*K. pneumoniae* Carbapenemase
LPS	Lipopolysaccharide
ETA	Endotracheal Aspirate
BAL	Bronchoalveolar Lavage
ICU	Intensive Care Unit
APACHE	Acute Physiology and Chronic Health Evaluation
DM	Diabetes Mellitus
HPT	Hypertension
MDR	Multi-drug Resistant
CLD	Chronic Liver Disease
TPN	Total Parenteral Nutrition
EVD	Extra Ventricular Device

ABSTRAK

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Objektif : Polymyxin adalah salah satu antibiotik yang telah lama diperkenalkan, dan kini digunakan kembali setelah sekian lama sebagai terapi infeksi kuman gram negatif, disebabkan oleh kurangnya penemuan antibiotik baru pada masa kini. Tujuan penyelidikan ini adalah untuk mengkaji hasil terapi dan mencari factor risiko terhadap kegagalan terapi polymyxin B di Unit Rawatan Rapi. Hasil terapi yang dilihat adalah kesembuhan secara klinikal dan kegagalan secara klinikal.

Metodologi : Kajian ini dijalankan secara 'crossectional', menggunakan rekod perubatan, dan dilaksanakan di Unit Rawatan Rapi, Hospital Universiti Sains Malaysia. Kajian ini melibatkan 96 kes jangkitan kuman gram negatif (jangkitan kuman di dalam darah, dan jangkitan paru-paru), di mana kuman penyebabnya adalah *Acinetobacter* spp, *Acinetobacter baumanii, Klebsiella pneumonia* and *Pseudomonas aeruginosa* yang diisolasi daripada specimen cecair endotrakea, darah, dan 'bronchoalveolar lavage'. Pemilihan sampel bermula dari senarai pesakit yang mendapat rawatan polymyxin B dari data farmasi dari tahun 2010-2014. Sebanyak 96 sample diambil secara rawak dan rekod perubatan kes-kes ini dilihat kembali. Data demografik, sejarah penyakit dahulu, penggunaan antibiotik, keputusan mikrobiologi dan hasilnya dicatat.

Keputusan : Daripada 96 sampel yang dikaji, sebanyak 51% (49 kes) menunjukkan kesembuhan selepas terapi polymyxin, manakala 49% atau 47 kes yang lainnya mengalami kegagalan. Kesemua kes kegagalan terapi berakhir dengan kematian, dan semua kematian tersebut adalah disebabkan oleh kuman gram negatif. Faktor risiko yang dikenalpasti menyebabkan kegagalan berdasarkan analisis 'Multiple Logistic Regression' adalah adanya jangkitan kuman di dalam darah (p=0.005) dan dos polymyxin yang tidak optimal (p=0.005). Polymyxin B dapat ditoleransi dengan baik oleh hampir semua subjek, di mana hanya 7 dari 96 sunjek sahaja menunjukkan penurunan fungsi ginjal, walaubagaimanapun penurunan fungsi ginjal ini tidak mengakibatkan penggunaan polymyxin B dihentikan.

Kesimpulan : Sebagai kesimpulan, kuman gram negatif yang resistan tehadap pelbagai antibiotik mempunyai kadar kematian yang semakin meningkat dan membimbangkan. Sensitiviti kuman yang pantas, antibiotik yang sesuai seperti polymyxin B dan diberikan dengan kadar segera, juga kombinasi antibiotik seperti sulperazone dapat menjanjikan kesembuhan terhadap pesakit-pesakit kritikal.

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demographic profiles, underlying diseases, potential risk factors, antibiotic usage, possible adverse effects, microbiology results and outcome were reviewed.

Results: Clinical outcome was evaluated for the 96 samples. Clinical cure contributed to 51% of the cases (49 cases) meanwhile another 47 cases (49%) contributed to clinical failure. Percentage of clinical cure was slightly higher compared to clinical failure for this study. 47 clinical failure subject (49.0%) reported death and all were referred to attributable mortality. Associated risk factors for polymyxin B treatment failure by Multiple Logistic Regression model were primary bacteremia (p=0.005) and inappropriate dose of polymyxin B (p=0.005). Polymyxin B was well tolerated by almost all of our sample, whereby only 7 out of 96 cases experienced deteriorating renal function, and it was not lead to discontinuation of the treatment..

Conclusions: In conclusion, mortality associated with multidrug resistant gram negative pathogens continues to be high. The early susceptibility, prompt and optimal antibiotic such as polymyxin B and also combination of antibiotic in particular with sulperazaone seems to have a survival benefit in this critically ill population.

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CHAPTER 1

INTRODUCTION

Multidrug-resistant Gram-negative organisms (MDRGNs) have developed as a major hazard to hospitalized patients. It have been associated with high mortality rates ranging from 30 to 70% (Tamma *et al.*, 2012). Gram-negative bacilli, particularly those with a high level of intrinsic resistance to many antibiotic classes and great ability to acquire resistance, such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Acinetobacter baumannii*, cause infections that are extremely difficult to treat (Kwa *et al.*, 2008).

Treatment of infections caused by these pathogens also has become considerably more challenging. It is due to the stagnation in development of novel antimicrobial agents to threat this pathogen. The emergence of these pathogen resistant to almost all antibiotics therefore has led to the re-administration of Polymyxins as "salvage" therapy in critically ill patient (Michalopoulos and Falagas, 2008).

Polymyxins were released in the late 1950's. However, the usage were decreased due to the potential toxicity and readily available of less toxic antibiotics. But later on, interestingly more clinical reports eventually demonstrated the tolerability, safety, and effectiveness of Polymyxins (Zavascki *et al.*, 2007).

There are five different types of polymyxins products (polymyxin A through E); however, only polymyxin B and polymyxin E (colistin) are used in clinical practice. Although polymyxin B and polymyxin E (colistin) have the same mechanism of action and the same pattern of resistance, colistin is more commonly used in clinical practice. There is also very limited clinical experience with Polymyxin B in the literature (Zavascki *et al.*, 2008).

There are few studies investigating the use of polymyxin B for treatment of infections caused by MDR Gram-negative bacilli, mostly *Acinetobacter spp*. and *Pseudomonas Aeruginosa*. Holloway *et al.* (2006) reported good results after administration of intravenous polymyxin B in 29 critically ill patients with infections caused by MDR *Acinetobacter baumannii*. The observed clinical cure was 76%, whereas crude mortality rate was 27%.

Pereira *et al.* (2007) described clinical features and outcomes of 19 patients treated with inhaled polymyxin B. Fourteen of them had nosocomial pneumonia and were concomitantly treated with iv polymyxin B. *Pseudomonas aeruginosa* was the aetiological agent in 11 of these 14 patients. Nine (64%) of the 14 patients died during hospitalization, although 13 (93%) of them were described as having a good clinical outcome of the pneumonia.

Dubrovskaya *et al.* (2013) describes outcomes of patients with CRKP infections treated with Polymyxin B monotherapy. Forty patients were included in the analysis. Twenty-nine of 40 (73%) patients achieved clinical cure as defined by clinician-documented improvement in signs and symptoms of infections, and 17/32 (53%)

patients with follow-up culture data achieved microbiological cure. The clinical cure rate achieved in this retrospective study was 73% of patients with CRKP infections treated with polymyxin B monotherapy.

This study was designed to determine the outcome of polymyxin B therapy for multi-drug resistant gram negative organisms and to identify risk factors for the treatment failure in critical care unit HUSM. This will help clinicians to gain clear picture regarding the outcome of Polymyxin B treatment and risk factors for treatment failure in our local situation since there are very large variations among regions, countries, hospitals and settings.

It is also hoped that the results will provide knowledge of the general risk factors associated with Polymyxin B treatment failure so that it may help avoid and/or recognise this complication of therapy at an early stage.

And finally it is expected that clinician's awareness regarding the treatment failure will be increased because awareness may potentially lead to improve outcomes.

CHAPTER 2

LITERATURE REVIEW

2.1 Multidrug-Resistant Gram Negative Bacili Infections

2.1.1 Definition

For epidemiologic purposes, in literal term, MDR means 'resistant to more than one antimicrobial agent', but no standardized definitions for MDR have been agreed so far (Magiorakos *et al.*, 2012). Many definitions are being used to characterize multidrug resistance patterns in gram-positive and gram-negative organisms, however there is still absence of specific definitions for MDR in clinical study protocols leads to difficulties to compae the data. Nevertheless, group of international experts came together through a joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), to create a standardized international terminology to describe acquired resistance profiles particularly in *Stapylococcus aureus, Enterococcus* spp, *Enterobactericiae* (other than *Salmonella* and *Shigella*), *Pseudomonas aeruginosa* and *Acinetobacter* spp. All are bacteria often responsible for healthcare-associated infections and prone to multidrug resistance. (Falagas *et al.,* 2006),(Cohen *et al.,* 2008). The experts agreed that three issues need to be considered to develop the definitions: (i) how to create antimicrobial 'categories' that would be epidemiologically meaningful; (ii) how to select the antimicrobial categories

and agents to be tested for each relevant bacterium; and (iii) how to define resistance within antimicrobial category (Magiorakos *et al.*, 2012).

Finally, the definitions for the characterization of bacterial isolates that are MDR, XDR (extensively drug-resistant) or PDR (pandrug-resistant) are proposed and given in Table 1. For all three definitions, non-susceptibility refers to either a resistant, intermediate or non-susceptible result obtained from in vitro antimicrobial susceptibility testing (Magiorakos *et al.*, 2012).

MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories, XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories), whereas PDR is defined as non-susceptibility to all agents in all antimicrobial categories (i.e. no agents tested as susceptible for that organism).

Table 2.1. Definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) Gram-negative bacteria

Bacterium	MDR	XDR	PDR
Enterobacteriaceae	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 22	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 2.2	Non-susceptibility to all agents in all antimicrobial categories for each bacterium in Tables 2.2–2.4
Pseudomonas aeruginosa	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 2.3	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 2.3	
***Acinetobacter* spp.**	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 2.4	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 2.4	

http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/ARHAI/Pages/public_consultation_clinical_microbiology_infection_article.aspx.

Table 2.2. Enterobacteriaceae; antimicrobial categories and agents used to define MDR, XDR and PDR

Antimicrobial category	Antimicrobial agent	Species with intrinsic resistance to antimicrobial agents or categories
Aminoglycosides	Gentamicin	*Providencia rettgeri (P. rettgeri), Providencia stuartii (P. stuartii)*
	Tobramycin	*P. rettgeri, P. stuartii*
	Amikacin	
	Netilmicin	*P. rettgeri, P. stuartii*
Anti-MRSA cephalosporins	Ceftaroline (approved only for Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca	
Antipseudomonal penicillins + b-lactamase inhibitors	Ticarcillin-clavulanic acid	*Escherichia hermannii (E. hermanii*
	Piperacillin-tazobactam	*E. hermanii*
Carbapenems	Ertapenem	
	Imipenem	
	Meropenem	
	Doripenem	
Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins	Cefazolin	*Citrobacter freundii (C. freundii), Enterobacter aerogenes (E. aerogenes), Enterobacter cloacae (E. cloacae), Hafnia alvei (H. alvei), Morganella morganii (M. morganii), Proteus penneri (P. penneri), Proteus vulgaris (P. vulgaris), P. rettgeri, P. stuartii, Serratia marcescens (S. marcescens)*
	Cefuroxime	*M. morganii, P. penneri, P. vulgaris, S.marcescens*
Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins	Cefotaxime or ceftriaxone	
	Ceftazidime	
	Cefepime	
Cephamycins	Cefoxitin	*C. freundii, E. aerogenes, E. cloacae, H. alvei*
	Cefotetan	*C. freundii, E. aerogenes, E. cloacae, H. alvei*
Fluoroquinolones	Ciprofloxacin	
Folate pathway inhibitors	Trimethoprim-sulphamethoxazole	
Glycylcyclines	Tigecycline	*M. morganii, Proteus mirabilis (P. mirabilis), P. penneri, P. vulgaris, P. rettgeri, P. stuartii*
Monobactams	Aztreonam	
Penicillins	Ampicillin	*Citrobacter koseri (C. koseri), C. freundii, E. aerogenes, E. cloacae, E. hermanii, H. alvei, Klebsiellae spp., M. morganii, P. penneri, P. vulgaris, P. rettgeri, P. stuartii, S. marcescens*
Penicillins + b-lactamase inhibitors	Amoxicillin-clavulanic acid	*C. freundii, E. aerogenes, E. cloacae, H. alvei, M. morganii, P. rettgeri, P. stuartii, S. marcescens*

	Ampicillin-sulbactam	*C. freundii, C. koseri, E. aerogenes, E. cloacae, H. alvei, P. rettgeri, S. marcescens*
Phenicols	Chloramphenicol	
Phosphonic acids	Fosfomycin	
Polymyxins	Colistin	*M. morganii, P. mirabilis, P. penneri, P. vulgaris, P. rettgeri, P. stuartii, S. marcescens*
Tetracyclines	Tetracycline	*M. morganii, P. mirabilis, P. penneri, P. vulgaris, P. rettgeri, P. stuartii*
	Doxycycline	*M. morganii, P. penneri, P. vulgaris, P. rettgeri, P. stuartii*
	Minocycline	*M. morganii, P. penneri, P. vulgaris, P. rettgeri, P. stuartii*

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Table 2.3. *Pseudomonas aeruginosa*; antimicrobial categories and agents used to define MDR, XDR and PDR

Antimicrobial category	**Antimicrobial agent**
Aminoglycosides	Gentamicin
	Tobramycin
	Amikacin
	Netilmicin
Antipseudomonal carbapenems	Imipenem
	Meropenem
	Doripenem
Antipseudomonal cephalosporins	Ceftazidime
	Cefepime
Antipseudomonal fluoroquinolones	Ciprofloxacin
	Levofloxacin
Antipseudomonal penicillins + b-lactamase inhibitors	Ticarcillin-clavulanic acid
	Piperacillin-tazobactam
Monobactams	Aztreonam
Phosphonic acids	Fosfomycin
Polymyxins	Colistin
	Polymyxin B

http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/ARHAI/Pages/public_consultation_clinical_microbiology_infection_article.aspx.

Table 2.4. *Acinetobacter* spp.; antimicrobial categories and agents used to define MDR, XDR and PDR

Antimicrobial category	Antimicrobial agent
Aminoglycosides	Gentamicin
	Tobramycin
	Amikacin
	Netilmicin
Antipseudomonal carbapenems	Imipenem
	Meropenem
	Doripenem
Antipseudomonal fluoroquinolones	Ciprofloxacin
	Levofloxacin
Antipseudomonal penicillins + b-lactamase inhibitors	Ticarcillin-clavulanic acid
	Piperacillin-tazobactam
Extended-spectrum cephalosporins	Cefotaxime
	Ceftriaxone
	Ceftazidime
	Cefepime
Folate pathway inhibitors	Trimethoprim-sulphamethoxazole
Penicillins + b-lactamase inhibitors	Ampicillin-sulbactam
Polymyxins	Colistin
	Polymyxin B
Tetracyclines	Tetracycline
	Doxycycline
	Minocycline

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2.1.2 Prevalance

Gram-negative bacilli are the primary cause of serious infections in both the community and hospital settings. Resistance to individual antimicrobials rapidly increases in gram-negative bacilli (D'Agata, 2004).

Rate of MDR, gram-negative bacilli has been reported in several studies. According to one of the studies, the global SENTRY Antimicrobial Surveillance Program reported percentage of MDR *Pseudomonas aeruginosa* in 1997 to 1999 ranged

from 0.9% in Canada to 8.2% in Latin America. In this study, MDR was defined as resistance to piperacillin, ceftazidime, imipenem, and gentamicin (Gales *et al.*, 2001).

In another study by the Surveillance Network Database–USA, it was reported that rate of MDR *Pseudomonas aeruginosa*, defined as resistance to at least three of the antimicrobials ceftazidime, ciprofloxacin, gentamicin, and imipenem, increased from 5.5% in 1998 to 7.0% in 2001, among non-ICU patients. The Surveillance Network also described that rate of multidrug resistance among members of the family Enterobacteriaceae in 2001, ranged from 0.3% for *Proteus mirabilis* to 3.1% *for Escherichia coli (Karlowsky et al., 2003).*

Meanwhile, a 9-year surveillance study by D'Agata (2004) testified that, a significant rise in multidrug resistance, defined as resistance to 3 or more antimicrobial classes from 1994 to 2002, was observed among gram-negative bacilli. Among all nosocomial isolates, multidrug-resistant *Pseudomonas aeruginosa* increased from 1% in 1994 and 1995 to 16% in 2002. Similar rises were noticed for members of the family *Enterobacteriaceae* with rate of multidrug-resistant *Klebsiella species*, multidrug-resistant *Proteus species*, and multidrug-resistant *Escherichia coli* increased from less than 1% in 1994 and 1995 to 17%, 9%, and 4%, respectively, in 2002. The most common coresistant antimicrobial pattern among these multidrug-resistant, gram-negative bacilli included quinolones, third-generation cephalosporins, and aminoglycosides. Whereas, ceftazidime was included among multidrug-resistant *Pseudomonas aeruginosa* isolates, in all co-resistant patterns (D'Agata, 2004).

2.2 Common organisms

2.2.1 General

Characteristically, gram-negative bacteria are non-pathogenic in the immunocompetent host, especially for the *Enterobacteriaceae* and the non fermentative gram-negative bacilli. However, gram-negative bacteria can become significant pathogens in the incapacitated host. The ability to prevent and treat gram-negative nosocomial infection is mostly troubled by antimicrobial resistance (Hidron *et al.*, 2008).

Due to higher mortality rate associated with these organisms worldwide, three common multidrug resistant gram-negative bacilli organisms will be discussed in this literature, namely *Acinetobacter* spp., *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*.

2.2.2 *Acinetobacter* spp.

2.2.2.1 Background

Acinetobacter is a gram-negative coccobacillus that has appeared as a vital nosocomial pathogen. It is non-motile, encapsulated, and non-fermentative. It belongs to the family of *Neisseriaceae (Rungruanghiranya et al., 2005). Acinetobacter baumannii, Acinetobacter genomic species 3,* and *Acinetobacter genomic species 13TU* are the predominant pathogenic organisms among all the *Acinetobacter* spp. Practically, these species are often grouped together using the term 'Baumannii group'. They

frequently cause persistent outbreaks within and across healthcare facilities, and also are proficient in developing resistance to multiple antimicrobial agents (28).

2.2.2.2 Epidemiology of MDR *Acinetobacter* spp.

The incidence of nosocomial infections caused by *Acinetobacter* spp. has increased gradually in recent years worldwide.

In the UK, extensive outbreaks of carbapenem-resistant *Acinetobacter* spp. have arisen since 2000. A number of different clones were identified affecting multiple health care institutions. The rates of non-susceptibility to meropenem rose from 13 to 29% between 2004 and 2008. In the meantime, non-susceptibility of *Acinetobacter* spp. to other classes of antimicrobials was reported at: aminoglycosides 20%; ciprofloxacin 30%; ceftazidime 70%; cefotaxime 89%; piperacillin/ tazobactam 50% in 2008. In European countries, the highest resistance rates have been reported in Mediterranean regions including Greece, Turkey, Italy and Spain (Coelho *et al.*, 2006).

Likewise in the US, data on healthcare-associated infections showed that 65-75% of *Acinetobacter* spp. isolates were multi-drug resistant, and that carbapenem non-susceptibility rose from 9% in 1995 to 57% in 2008 (Hoffmann *et al.*, 2010). Figures from Ireland on *Acinetobacter* is somewhat limited. A university hospital in Ireland identified 114 *Acinetobacter* spp. isolated from clinical specimens over a 30 month period between 2005 and 2007. Automated methods recognized 77 as *A. baumannii*, however with molecular methods, the major species was actually *A. genomic species 3*. Out of 114 isolates, 11% were carbapenem resistant (Boo *et al.*, 2009).

2.2.2.3 Clinical Significance

It is observed that Ventilator-associated pneumonia (VAP) is the most common infection due to *Acinetobacter*. Rates of VAP due to *Acinetobacter* approach 5-10% in some countries. *Acinetobacter* species are known causes of infection in patients with surgical sites, burn, wounds, and lately have been recognised in infections complicating injured military personnel. *Acinetobacter* species cause BSIs in minority in the United Kingdom and in the United States. Nevertheless, crude mortality figures attribute to *Acinetobacter* infection differ considerably ranging from 34-67%. This is due to inadequate empirical therapy when managing infections. Colonisation and infection have also been independently associated with higher morbidity, costs and prolonged hospitalisation (Garnacho-Montero *et al.*, 2005).

2.2.2.4 Laboratory Detection

Acinetobacter species grow well routinely on standard culture media. They can be identified at the genus level (Gram-negative, catalase-positive, oxidase negative and non-fermenting coccobacilli). Accurate subspeciation remains challenging and lengthy. Automated methods are often unable to distinguish between the three species of clinical significance. Furthermore, phenotypic methods to identify mechanisms of resistance are unreliable. Hence, there has been a growing use of molecular methods both for speciation, and for detection of particular resistance gene determinants. Such methods are also important for epidemiological purposes in an outbreak setting (28).

2.2.3 *Klebsiella pneumonia*

2.2.3.1 Background

Klebsiella pneumoniae is a gram-negative bacilli that normally live in the gastrointestinal tract. There is a term used to define this group of organism, called Enterobacteriaceae. Other organisms include in Enterobacteriaceae group are *Escherichia coli, , Enterobacter cloacae*, and *Citrobacter freundii*. β-lactams are a group of antimicrobials that contain some of the most frequently used antibiotics (such as penicillins, cephalosporins, monobactams and carbapenems) for treating infections.
The main mechanism for the development of resistance to the various types of β-lactam antimicrobials is the production of enzymes, known as b-lactamases by Enterobacteriaceae. Nowadays, lots of b-lactamases exist, including extended spectrum b-lactamases (ESBL), AmpC b-lactamases and carbapenemases. These enzymes have multiple ranges of hydrolytic activity and are usually located on mobile genetic elements, known as plasmids, that can enhance their transmission ability (Bradford, 2001).

2.2.3.2 Epidemiology

Carbapenem resistance, caused by the combination of ESBL/AmpC production and porin loss, has been reported for several years. Although resistant strains can be

transmitted between patients, this resistance mechanism cannot be transferred to other bacterial strains (28).

Carbapenemases are a different group of broad spectrum b-lactamases. The most common encountered carbapenemases are:

- Klebsiella pneumoniae carbapenemase (KPC)
- New Delhi metallo-b-lactamase (NDM)
- Verona Integron-encoded metallo-β-lactamase (VIM)
- Oxacillinase (OXA)

The most worrying part is the rapid international dissemination of carbapenemases, as illustrated by the importation of NDM-1 from the Indian subcontinent to the United Kingdom and other European countries as well as the global importation of KPC from the United States to various continents. The fast spread of these carbapenemases is usually facilitated by transfer of plasmids between strains or species and/or clonal dissemination of certain strains (Nordmann *et al.*, 2009; Kumarasamy *et al.*, 2010).

In Europe, Greece is considered endemic for CRE. Significant problems of CRE dissemination have also been reported in other European countries such as Italy, Poland, France, Spain and the UK (Nordmann *et al.*, 2009; Kumarasamy *et al.*, 2010).

Although mainly found in patients from the UK, NDM-1-producing Enterobacteriaceae have also been reported in other European countries such as Germany, France and Scandinavian countries. OXA-48 has been reported in various

regions including the India, Europe, Middle East, and North Africa (Grundmann *et al.*, 2010).

First data on carbapenem resistance among *K. pneumoniae* isolates were stated from EARSS/ EARS-NET in 2005, when Greece already reported 28% carbapenem resistance. Greece reported 49% carbapenem resistance among *K. pneumoniae* isolates in 2010. Importantly in 2010 , Italy's resistance rate increased from 1.3% in 2009 to 15%.

CRE have also been encountered in Irish healthcare centers since 2009. While only sporadic cases had been described in 2009 and 2010, the epidemiology of CRE changed significantly in Ireland in 2011. Throughout 2011, CRE was reported from 36 patients in eight Irish hospitals, with four hospitals reporting CRE outbreaks. In January 2011, an outbreak with KPC producing *K. pneumoniae* was reported in the mid-west, with documented interhospital spread. An outbreak of OXA-48 *K. pneumoniae* occurred in a tertiary hospital in Dublin during spring 2011. Other hospitals have also reported intermittent cases of KPC-, OXA-48-, and VIM-producing *K. pneumoniae* as well as VIM-producing *E. cloacae* (Boo *et al.*, 2009). The first incident of NDM-1 producing *K. pneumoniae* discovered in Ireland was notified in summer 2011. In June 2011, a monthly prevalence survey was conducted in 40 Irish critical care units. Patients were screened weekly for rectal carriage of carbapenemase-producing CRE. CRE was not detected in any of the 40 participants during this study (Prior *et al.*, 2010; O'Brien *et al.*, 2011).

2.2.3.3 Clinical Significance

Members of the Enterobacteriaceae group frequently cause bacterial infections in all ages patients. The most common sites of infection are UTI, intra-abdominal sepsis, surgical site infections and BSI (28).

There are less therapeutic options for the treatment of infections caused by resistant Enterobacteriaceae. This is because of these organisms are often resistant to other classes of antimicrobials such as aminoglycosides and fluoroquinolones. At present, carbapenems are the treatment of choice for infections caused by ESBL-producing and AmpC-hyperproducing organisms. However it is afraid that the reliance to carbapenems will lead to the emergence of carbapenem resistance (Bradford, 2001).

Most *Enterobacteriaceae* producing carbapenemases are resistant to carbapenems in vivo . Therefore therapeutic options for CRE infection are very limited. The resistance profiles of most strains leave only a few antimicrobial agents available as potential therapeutic options for example tigecycline, fosfomycin and colistin. However, non-susceptibility or resistance to these antimicrobials is also reported progressively in CRE (Nordmann *et al.*, 2009; Kumarasamy *et al.*, 2010). Some CRE strains may have carbapenem minimum inhibitory concentrations (MICs) that fall within the susceptible range according to CLSI or EUCAST breakpoint criteria. Yet, the clinical significance of carbapenemases in such strains is still unclear. Infections caused by resistant *Enterobacteriaceae* are associated with increase risk of mortality. Mortality rates associated with infections caused by CRE ranged from 38-57% (Boo *et al.*, 2009).

2.2.3.4 Laboratory Detection

Optimal laboratory detection of resistant Enterobacteriaceae from speciments is essential for therapeutic decisions as well as for timely and effective implementation of infection control measures. As therapeutic options can be very limited, (particularly in the case of CRE), it is recommended that every effort should be undertaken by laboratories to identify resistant Enterobacteriaceae (28).

In some laboratories, carbapenem susceptibility testing may not be regularly performed in Enterobacteriaceae isolates from certain clinical samples, mainly urine specimens. Remarkably, a significant proportion of isolates from patients with CRE were from urine specimens. A European working group has recently recommended susceptibility testing of Enterobacteriaceae from all anatomical sites with at least one carbapenem (Grundmann *et al.*, 2010). Most carbapenemase-producing Enterobacteriaceae are similarly resistant to cephalosporins. The potential pitfall of using cephalosporin (such as cefpodoxime) as a substitute indicator for carbapenem resistance is the failure to detect OXA-48-producing strains, which can be susceptible to cephalosporins unless co-producing ESBLs (Nordmann *et al.*, 2009; Kumarasamy *et al.*, 2010).

Several phenotypic tests have been described for the detection of carbapenemase production in Enterobacteriaceae. Two most frequently used methods are the modified Hodge test (MHT) and inhibitor-based synergy tests.. These phenotypic tests can be used by laboratories to further analyse organisms with elevated carbapenem MICs for potential carbapenemase production. As none of the above phenotypic methods have

universally accepted interpretive standards, the use of molecular methods such as end-point or real-time PCR for the detection of carbapenemase genes has been recommended for isolates suspected of carbapenemase production (Miriagou *et al.*, 2010).

2.2.4 *Pseudomonas aeruginosa*

2.2.4.1 Background

Pseudomonas aeruginosa is a Gram-negative bacteria existing widely in the environment, present in diverse environmental settings (e.g. aquatic environments and soil) and is also known to colonise plants, animals and humans. *P. aeruginosa* is mainly termed as an opportunistic pathogen causing disease in compromised hosts, for example immunocompromised patients, patients in intensive care settings, and patients with chronic lung disease (28).

2.2.4.2 Epidemiology

P. aeruginosa represents a considerably important nosocomial pathogen. Due to the high prevalence of infection, increasing rates of antimicrobial non-susceptibility and the worrying characteristic of the emergence of resistance during therapy further interrupt efforts to successfully control infections due to this pathogen. Non-susceptibility rates of *P. aeruginosa* to many classes of antimicrobials have stayed

largely stable across Europe and the US; however a worrying trend of increasing non-susceptibility to carbapenems has been observed worldwide (28).

The frequency of MDR *P. aeruginosa* amongst BSI isolates in Europe in 2010, was estimated to be 15%. The highest rates of MDR *P. aeruginosa* were reported from Greece (42.5%), and the Czech Republic (29%). This remains highest in Southern and Eastern European states. Overall 16 of 28 countries reported that 10% or more of the *P. aeruginosa* isolates were resistant to carbapenems. Countries with the highest non-susceptibility to these agents include Cyprus (29%), Bulgaria (31%) and Greece (43%), (28).

Data from BSI surveillance in the UK between 2001 and 2006 reported that 2.5 - 4% of all BSI isolates were *P. aeruginosa*. Non-susceptibility rates remained broadly stable with the exception of carbapenems. Non-susceptibility to meropenem increased from 5.7% to 10%. Isolates from an ICU setting showed statistically higher rates of non-susceptibility to imipenem and piperacillin/tazobactam. US estimates of MDR amongst *P. aeruginosa* raised at 18% in 2000, 21% in 2003, and most recently 17% in 2008 (28).

2.2.4.3 Clinical Significance

P. aeruginosa is the second most common cause of healthcare-associated pneumonia in the US, causing 14-16% of cases (30). European surveillance data for ICUs showed *P. aeruginosa* as the causative pathogen for 23-30% of cases of VAP, 19% of UTI and 10% of BSI. Equivalent figures from ICUs in the US are VAP 21%,

UTI 10%, and BSI 3% respectively. Infection due to MDR *P. aeruginosa* is associated with increased morbidity and mortality, prolonged length of stay, and increased costs. In particular, inappropriate empirical therapy in the context of MDR infection has been independently associated with both prolonged bacteraemia and higher morbidity and mortality (Hoffmann *et al.*, 2010).

2.2.4.4 Laboratory Detection

P. aeruginosa is easily isolated and identified in the laboratory. Antimicrobial resistance can be measured using standard disc diffusion or commercial automated methods. Molecular methods have been used to identify specific resistance mechanisms. By benefit of the relative impermeability of its outer membrane, *P. aeruginosa* is intrinsically resistant to many antimicrobials. Moreover, multiple separate resistance mechanisms have been identified, which combined contribute to the resistant phenotypes observed in clinical settings. The mechanisms may be inherent to the bacterium or acquired via mobile genetic elements/plasmids (28).

2.3 Frequent types of infection caused by MDR GN Infections

The gram-negative bacilli differ in the frequencies that they cause the 4 most common types of hospital-acquired infection: bloodstream infection (BSI), pneumonia, surgical site infection (SSI), and urinary tract infection (UTI) (Weinstein *et al.*, 2005). During the past 20 years, changes in health care, infection-control practices, and

antimicrobial use and resistance may have influenced the frequency that these gram-negative organisms are associated with hospital-acquired infection.

In this literature, all the 4 types of infections will be discussed including definition and criteria for diagnosing based on CDC/NHSN Surveillance Definition of Healthcare-Associated Infection and Criteria for Specific Types of Infections in the Acute Care Setting. However in this study only 2 types of the infection are included, namely BSI and pneumonia.

2.3.1 Blood Stream Infection

Primary bloodstream infection includes laboratory-confirmed bloodstream infection and clinical sepsis. The definition of clinical sepsis is intended primarily for infants and neonates (Garner *et al.*, 1988; Horan *et al.*, 2008).

Laboratory-confirmed bloodstream infection must meet one of the following criteria:

a. Recognized pathogen isolated from blood culture AND pathogen is not related to infection at another site.

b. One of the following: fever (>38^{o} C), chills, or hypotension AND any of the following:

   i. Common skin contaminantt isolated from two blood cultures drawn on separate occasions AND organism is not related to infection at another site.

ii. Common skin contaminant isolated from blood culture from patient with intravascular access device AND physician institutes appropriate antimicrobial therapy.

iii. Positive antigen test on blood AND organism is not related to infection at another site.

c. Patient <12 months of ages has one of the following: fever (>38^{o} C), hypothermia (<37^{o} C), apnea, or bradycardia AND any of the following:

i. Common skin contaminant isolated from two blood cultures drawn on separate occasions AND organism is not related to infection at another site.

ii. Common skin contaminant isolated from two blood cultures drawn on separate occasion and organism is not related to infection at another site.

iii. Positive antigen test on blood and pathogen is not related to infection at another site.

Clinical sepsis must meet either of the following criteria:

a. One of the following clinical signs or symptoms with no other recognized cause: fever (>38^{o} C), hypotension (systolic pressure <90 mm Hg), or oliguria (<20 ml/hr) AND all of the following:

i. Blood culture not done or no organism or antigen detected in blood.

ii. No apparent infection at another site.

iii. Physician institutes appropriate antimicrobial therapy for sepsis.

b. Patient <12 months of age has one of the following clinical signs or symptoms with no other recognized cause: fever (>38^{o} C), hypothermia (<37^{o} C), apnea, or bradycardia AND all of the following:

   i. Blood culture not done or no organism or antigen detected in blood.

   ii. No apparent infection at another site.

   iii. Physician institutes appropriate antimicrobial therapy for sepsis.

When an organism isolated from blood culture is compatible with a related nosocomial infection at another site, the bloodstream infection is classified as a secondary bloodstream infection. Exceptions to this are intravascular device-associated bloodstream infections, all of which are classified as primary even if localized signs of infection are present at the access site (Garner *et al.*, 1988; Horan *et al.*, 2008).

2.3.2 Pneumonia

Pneumonia is defined separately from other infections of the lower respiratory tract. The criteria for pneumonia involve various combinations of clinical, radiographic, and laboratory evidence of infection. In general, expectorated sputum cultures are not useful in diagnosing pneumonia but may help identify the etiologic agent and provide useful antimicrobial susceptibility data. Findings from serial chest x-ray studies may be more helpful than those from a single x-ray film (Garner *et al.*, 1988; Horan *et al.*, 2008).

Pneumonia must meet one of the following criteria:

a. Rales or dullness to percussion on physical examination of chest AND any of the following:

   i. New onset of purulent sputum or change in character of sputum.

   ii. Organism isolated from blood culture.

   iii. Isolation of pathogen from specimen obtained by transtracheal aspirate, bronchial brushing, or biopsy.

b. Chest radiographic examination shows new or progressive infiltrate, consolidation, cavitation, or pleural effusion AND any of the following:

   i. New onset of purulent sputum or change in character of sputum.

   ii. Organism isolated from blood culture.

   iii. Isolation of pathogen from specimen obtained by transtracheal aspirate, bronchial brushing, or biopsy.

   iv. Isolation of virus or detection of viral antigen in respiratory secretions.

   v. Diagnostic single antibody titer (IgM) or fourfold increase in paired serum samples (IgG) for pathogen.

   vi. Histopathologic evidence of pneumonia.

c. Patient <12 months of age has two of the following: apnea, tachypnea, bradycardia, wheezing, rhonchi, or cough AND any of the following:

   i. Increased production of respiratory secretions.

   ii. New onset of purulent sputum or change in character of sputum.

   iii. Organism isolated from blood culture.

   iv. Isolation of pathogen from specimen obtained by transtracheal aspirate, bronchial brushing, or biopsy.