TITLE

THE CLINICAL AND MOLECULAR CHARACTERIZATION OF EXTENSIVELY DRUG RESISTANT (XDR) *ACINETOBACTER BAUMANNII* COMPLEX FROM CLINICAL ISOLATES IN HOSPITAL RAJA PEREMPUAN ZAINAB II

By:

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‘Indeed, in the creation of the heavens and the earth and the alternation of the night and the day are signs for those of understanding. Who remember Allah while standing or sitting or [lying] on their sides and give thought to the creation of the heavens and the earth, [saying],

"Our Lord, You did not create this aimlessly; exalted are You [above such a thing]; then protect us from the punishment of the Fire.”

(Ali ‘Imran 3:190-191)

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABBREVIATIONS

ANDRA	amplified ribosomal DNA restriction analysis
APACHE II	Acute Physiology and Chronic Health Evaluation II
ATCC	American Type Culture Collection
BHI	brain heart infusion
CAUTI	catheter-associated urinary tract infections
CLSI	Clinical Laboratory Standard Institute
CMS	colistimethate sodium
CRAB	carbapenem-resistant *Acinetobacter baumannii*
DNA	deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EDTA	Ethylenediaminetetraacetic acid
ESBL	Extended Spectrum β-Lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FITR	Fourier transform infrared spectroscopy
HAI	Healthcare-associated infection
HCL	Hydrochloric acid
HCW	healthcare workers
HDW	high dependency ward
HRPZ II	Hospital Raja Perempuan Zainab II
ICU	Intensive care units
IS	insertion sequence
IL-8	interleukin-8
LPS	lipopolysaccharides

MALDI-TOF MS	matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MBLs	Metallo-β-lactamases
MIC	minimum inhibitory concentration
MDR	Multidrug resistant
NLS	nuclear localization signal
NHSN	National Healthcare Safety Network
OMP	outer membrane protein
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
TBE	Tris Borate EDTA
TLR-4	Toll-like receptor 4
TNF	tumour necrosis factor
TSA	Trypton soy agar
TSB	Trypton soy broth
VAP	ventilator-associated pneumonia
XDR	extensive-drug resistant

ABSTRACT

THE CLINICAL AND MOLECULAR CHARACTERIZATION OF EXTENSIVELY DRUG RESISTANT (XDR) *ACINETOBACTER BAUMANNII* COMPLEX FROM CLINICAL ISOLATES IN HOSPITAL RAJA PEREMPUAN ZAINAB II

Introduction: *Acinetobacter baumannii (A. baumannii)* is an important cause of hospital-acquired infections. However, treating these infections is a challenge due to the isolation of multiply drug resistant strains that has limited antibiotic susceptibility. This study aims to consider the molecular characteristics of the XDR *A. baumannii*, the clinical characteristics of patients who are treated for the bacterial infection and recognize factors associated with poor treatment outcome.

Methodology: A cross sectional study was conducted among patients from which XDR *A. baumannii* were isolated from 1st January 2016 to 30th April 2017 in Hospital Raja Perempuan Zainab II. The isolates from non-repeat clinical samples which show extensive drug resistance pattern were collected and their species identification were confirmed using Vitek 2®. These isolates were then subjected to multiplex PCR analysis for the detection of oxacillinases resistance genes, namely $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$. Patients' records of infective cases were reviewed for demographic data and clinical characteristics. These data were analysed using multiple logistic regression to identify variables that lead to end-of-treatment mortality among patients who were infected with XDR *A. baumannii*.

Results: XDR *A. baumannii* were isolated from 116 patients, and 65 of these cases were considered infectives and were treated for their XDR *A. baumannii* infection. All the isolates harboured the $bla_{\text{OXA-51-like}}$ gene, while the $bla_{\text{OXA-23-like}}$ were detected in all

isolates but two. Most of the isolates regarded as clinically significant are from tracheal aspirate (45 samples), followed by blood (9 samples) and the remaining are from urine, tissue and pus (11 samples). Among those who are treated, the end-of-treatment mortality rate was 60.0% (39 patients). Repeat cultures after 72-hour of treatment were obtained from 31 of the infected patients, with 35.5% (11 patients) achieving microbiological eradication, 6.5% (2 patients) having microbiological noneradication while the majority (58.1%; 18 patients) having breakthrough infection. In the multivariable analysis, presence of sepsis (adjusted odds ratio (aOR) 7.93; 95% confidence interval (CI) 1.45, 43.26; *p*-value = 0.017) and presence of arterial catheter (aOR 15.53; CI 1.66, 145.71; *p*-value = 0.016) were independently associated with increased risk of end-of-treatment mortality. Conversely, longer treatment duration with polymyxin (aOR 0.71; CI 0.588, 0.856; *p*-value <0.001) was associated with decreased risk of end-of-treatment mortality.

Conclusions: Most of the XDR *A. baumannii* isolates in this study harboured the $bla_{\text{OXA-51-like}}$ gene and the $bla_{\text{OXA-23-like}}$. Almost half of the isolates were considered clinically significant. Among patients who are treated for XDR *Acinetobacter baumannii* infection, presence of sepsis and presence of arterial catheter significantly increased risk of mortality. Yet, the risk for end-of-treatment mortality may be reduced with longer duration of colistin therapy.

ABSTRAK

CIRI-CIRI MOLEKULAR DAN KLINIKAL *ACINETOBACTER BAUMANNI* KERINTANGAN ANTIBIOTIK MENYELURUH (XDR) DARIPADA SAMPEL KLINIKAL DARI HOSPITAL RAJA PEREMPUAN ZAINAB II

Pengenalan: *Acinetobacter baumannii* ialah salah satu bakteria penyebab utama jangkitan yang diperolehi semasa menerima rawatan di hospital. Walaupun begitu, merawat jangkitan ini kini menjadi semakin sukar disebabkan kewujudan bakteria rintang pelbagai antibiotik yang mempunyai tahap kepekaan antibiotik yang terhad. Kajian ini bertujuan untuk mengenalpasti ciri-ciri molekular *Acinetobacter baumanni* kerintangan antibiotik menyeluruh (XDR), ciri-ciri klinikal pesakit yang dijangkiti bakteria ini dan faktor-faktor yang berkaitan dengan kesudahan tidak baik setelah rawatan.

Tatacara Kajian: Satu kajian keratan rentas telah dijalankan di kalangan pesakit yang didapati mempunyai XDR *A. baumannii* dari sampel klinikal bermula 1 Januari 2016 sehingga 30 April 2017 di Hospital Raja Perempuan Zainab II. Bakteria daripada sampel klinikal bukan-berulang yang menunjukkan corak kerintangan antibiotik menyeluruh dikutip dan pengenalan spesis dilakukan menggunakan Vitek 2®. Bakteria ini kemudiannya dianalisa menggunakan PCR multipleks untuk mengesan gen kerintangan *oxacillinases*, iaitu $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ dan $bla_{\text{OXA-58-like}}$. Rekod pesakit-pesakit yang dijangkiti disemak untuk mendapatkan maklumat demografik dan klinikal. Maklumat ini dianalisa menggunakan logistik regresi berganda bagi mengenalpasti penentu yang berkaitan dengan mortaliti setelah rawatan bagi pesakit yang dijangkiti XDR *A. baumannii*.

Keputusan: XDR *A. baumannii* ditemui daripada 116 pesakit, dan 65 daripada pesakit ini dijangkiti dan dirawat untuk jangkitan XDR *A. baumannii*. Kesemua bakteria tersebut didapati mempunyai gen $bla_{\text{OXA-51-like}}$, dan $bla_{\text{OXA-23-like}}$ turut dikesan pada hampir kesemua bakteria kecuali dua. Kebanyakan bakteria yang dianggap signifikan dari segi klinikal dijumpai dari sampel cecair trakea (45 sampel), diikuti dengan sampel darah (9 sampel) dan yang lainnya daripada sampel air kencing, tisu dan nanah (11 sampel). Di kalangan mereka yang dirawat, mortaliti setelah rawatan adalah sebanyak 60% (39 pesakit). Kultur ulangan setelah 72 jam menerima rawatan diperolehi daripada 31 orang pesakit yang dijangkiti, dengan 35.5% (11 pesakit) berjaya mencapai pembasmian mikrobiologi, 6.5% (2 pesakit) gagal mencapai pembasmian mikrobiologi manakala majoriti (58.1%; 18 pesakit) mendapat jangkitan sampingan. Daripada analisa logistik regresi berganda, keadaan sepsis (nisbah kemungkinan terlaras (aOR) 7.93; julat keyakinan 95% (CI) 1.45, 43.26; nilai p = 0.017) dan kewujudan kateter arteri (aOR 15.53; CI 1.66, 145.71; nilai p = 0.016) dikaitkan secara bebas dengan peningkatan risiko mortaliti setelah rawatan. Tetapi, jangka masa rawatan dengan *polymyxin* yang lebih lama (aOR 0.7I; CI 0.588, 0.856; nilai p <0.001) dikaitkan dengan pengurangan risiko mortaliti setelah rawatan.

Kesimpulan: Kebanyakan XDR *A. baumannii* dalam kajian ini mengandungi gen $bla_{\text{OXA-51-like}}$ dan $bla_{\text{OXA-23-like}}$. Hampir separuh daripada bakteria yang dikutip dianggap signifikan dari segi klinikal. Di kalangan pesakit yang dirawat untuk jangkitan XDR *A. baumannii*, keadaan sepsis dan kewujudan kateter arteri meningkatkan risiko mortaliti secara signifikan. Walau bagaimanapun, risiko mortaliti setalah rawatan dapat dikurangkan dengan jangka masa rawatan *polymyxin* yang lebih lama.

1 Introduction

1.1 Nomenclature of *Acinetobacter* spp.

The first account of *Acinetobacter* spp. was in 1911 when a Dutch microbiologist describe an organism named *Micrococcus calco-aceticus* isolated from soil in a calcium-acetate-containing minimal medium (Beijerinck, 1911). Since then, similar organisms had been described and allocated to at least 15 distinct genera and species until the current genus *Acinetobacter* was proposed by Brisou and Prevot in 1954. The genus became widely accepted in 1968 after a survey by Baumann et al. showed that all the different species belonged to the *Acinetobacter* genus (Baumann *et al.*, 1968). Currently, the genus comprises of Gram-negative, non-fermenting, strictly aerobic, non-fastidious, non-motile, catalase-positive, oxidase-negative bacteria with a DNA G+C content of 39% to 47%. Another significant discovery in the *Acinetobacter* genus was achieved in 1986 by Bouvet and Grimont when they successfully distinguished 12 genospecies via DNA-DNA hybridization studies (Bouvet and Grimont, 1986). Some of these species were given formal species names, including *A. baumannii* (genomic species 2), *A. calcoaceticus* (genomic species 1), *A. haemolyticus* (genomic species 4), *A. johnsonii (*genomic species 7*), A. junii* (genomic species 5*),* and *A. lwoffi* (genomic species 8/9). *Acinetobacter* genomic species 3 and genomic species 13TU were later formally named as *A. pittii* and *A. nosocomialis* (Nemec et al., 2011). With the advent of molecular techniques, increasing number of distinct species within the *Acinetobacter* genus had been identified and 55 species had been named as of July 2017 (Parte, 2017).

A. calcoaceticus, A. baumannii, A. pittii, and *A. nosocomialis* are interrelated and cannot be distinguished from one another by phenotypic characteristics, thus it has

been proposed to refer to these species as the *A. calcoaceticus-A. baumannii* complex (Gerner-Smidt *et al.*, 1991). Another species had been recently identified among this complex: *A. seifertii* (Nemec *et al.*, 2015) and a provisional genomospecies called "between 1 & 3'. The problem with grouping these species together is that not all of them are clinically significant. *A. baumannii, A. pittii, A. nosocomialis* and *A. seifertii* are considered significant and had been implicated in human infections, but *A. calcoaceticus* is ubiquitous in soil and water and rarely been associated with serious clinical disease. This highlighted the need to distinguish between the *Acinetobacter* sp. within *A. baumannii* complex because these species differ in term of infectious potential, antimicrobial susceptibility and mortality rates, hence precise identification can guide therapy and limit unnecessary treatment of non-pathogenic strains.

1.2 Epidemiology

Acinetobacter spp. was initially regarded as a commensal opportunist, a pathogen of low virulence with minimal significance. However, the increasing use of complex invasive medical intervention in the last few decades had caused a surge in the frequency and severity of *Acinetobacter* infection. In the United States, data from the National Healthcare Safety Network (NHSN) from 2011 to 2014 showed *Acinetobacter* spp. accounted for 1.07% of the total isolates that cause healthcare-associated infection (HAI), with multidrug resistant strains accounting for 33-75% of the isolates, depending on samples (Weiner *et al.*, 2016). Data from European Centre for Disease Prevention and Control (ECDC) which conducted surveillance on HAI in intensive care units (ICU) in Europe in 2014 found that *Acinetobacter* spp. accounted for 2.5% and 1.6% of blood stream infection and urinary tract infection, respectively (ECDC, 2016), with carbapenem-resistance detected in 63.5% of *A. baumannii* isolates. In comparison, *Acinetobacter* spp. cause a much higher proportion of HCAI in developing countries

and may become the principal nosocomial pathogen. In South America, *Acinetobacter* spp. was the most prevalent isolate encountered from ICU patients with HCAI especially ventilator-associated pneumonia (VAP). It was responsible for up to 31% of the total isolates and the carbapenem resistance ranged from 56 to 76% (Luna *et al.*, 2014). Data from Africa showed that *Acinetobacter* spp. accounts for about 14.8% of ICU infections (Vincent *et al.*, 2009).

In the Southeast Asia region, the prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) ranges from 25% to 90.5% (Kiratisin *et al.*, 2012). In Malaysia, the rate of CRAB in 2016 was 60% (unpublished National Antibiotic Surveillance report 2016). The high prevalence of carbapenem resistance amongst important nosocomial pathogens warrants rigorous infection control measures and appropriate antimicrobial use in the Southeast Asia region.

1.3 Transmission

Acinetobacter spp. intrinsic resistance to desiccation leads to its environmental tenacity and high transmissibility. The pathogen thrives in hot and humid tropical climates, which may explain the high prevalence seen in hot and tropical countries (i.e. Africa, South America). Cases also show seasonal predilection; the rate of *Acinetobacter* infections in the United States increased by 54% between July and October compared to November through June (McDonald *et al.*, 1999).

In the healthcare setting, it is established that *Acinetobacter* spp. is transmitted to patients through transient colonization of the hands of the health care workers and persistence on environmental surfaces (Russotto *et al.*, 2015). Studies had also shown that the pathogen can be spread via aerosolization from infected and colonized patients (Spellberg and Bonomo, 2013). These aerosolized bacteria may

survive up to several weeks in the air (Yakupogullari *et al.*, 2016). However, a study had found that the frequency of air contamination by *A. baumannii* is variable. Factors that could reduce the rate of air contamination include the use of closed circuit mechanical ventilation system, frequent air exchanges and the bacterial burden of the patient, which may be low if the patient was treated with appropriate antibiotic (Rock *et al.*, 2015). The findings that suggest *Acinetobacter* could be transmitted via airborne route implicate that current practice of cleaning environmental surfaces episodically to prevent spread might not be sufficient. Efforts should also be made to disinfect the air in patients' rooms to ensure that the risk of transmission is reduced.

1.4 Pathogenesis

1.4.1 Virulence Factors

A. baumannii intrinsically has more human virulence potential than other *Acinetobacter* spp.; it grew better at 37°C and was better able to resist macrophage uptake compared to other species (Tayabali *et al.*, 2012). Even in the clinical context, a case-control study had shown that patients with *A. baumannii* bacteraemia has a higher 28-day mortality (37% vs 6%) than those with *A. ursingii* bacteraemia, even though multidrug resistance and inadequate initial therapy were as likely to occur in patients infected with either species (Chiu *et al.*, 2015).

Under dry conditions, *A. baumannii* adapts morphological changes such as thicker cell walls which may be a factor for its persistence on environmental surfaces (Houang *et al.*, 1998). Another important factor in the pathogen ability to resist desiccation is the Rec A enzyme which also mediates bacterial DNA repair and prevents its killing inside macrophages, thus contributing to its lethality in mice infection model (Aranda *et al.*, 2011). *Acinetobacter* sp. is resistant to disinfection, too; a study had

shown that the presence of ethanol alters its cell physiology, enabling *Acinetobacter* species to withstand the toxic effects of salt and allowing growth on culture media (Smith *et al.*, 2004). Apart from that, the presence of ethanol increased the production of proteins involved in lipid and carbohydrate anabolism and this correlates with increased carbohydrate biofilm content, enhanced biofilm formation on abiotic surfaces and decrease bacterial motility on semi-solid surfaces (Nwugo *et al.*, 2012).

The capsule of *A. baumannii* plays a key role in the defence of the pathogen and its virulence. The K1 capsular polysaccharide-positive strains showed optimal growth in human ascites fluid and human serum as compared to capsule-negative strains, suggesting the protective role the capsule plays against complement-mediated destruction, opsonization and phagocytic uptake (Russo *et al.*, 2010). Another study had also shown that exposure to antibiotics leads to hyperproduction of the capsular polysaccharide which in turn causes increased resistance to killing by host complement and increased virulence in a mouse model of systemic infection (Geisinger and Isberg, 2015). Glycoproteomic data revealed prevalence of O-linked glycoproteins, which is required for protein glycosylation and capsular polysaccahride production in *A. baumannii*. The O-linked glycosylation favors short (three to five residue) glycans with limited branching containing negatively charged sugars (Scott *et al.*, 2014).

One of the bacterial protein that has been widely implicated in the virulence of *A. baumannii* is the outer membrane protein A (OmpA). OmpA is the most abundant surface protein of the bacteria and study had shown that this protein binds to eukaryotic cells and translocates to the nucleus by a novel monopartite nuclear localization signal (NLS). This nuclear translocation induces cell cytotoxicity *in vitro* (Choi *et al.*, 2008). OmpA also plays a vital role in the pathogen ability to evade host's complement attack by binding factor H from human serum, thus regulating the alternative complement

pathway (Kim *et al.*, 2009). *Acinetobacter nosocomialis*, another species within the *A. baumannii* complex, also employed OmpA in its pathogenesis. Mutant strain of *A. nosocomialis* which are devoid of OmpA showed reduced adherence to A549 (human lung carcinoma) cells, which suggest that the protein played a significant role in bacterial adhesion to host epithelial cells (Kim *et al.*, 2016a). In a lethal model of infection of *A. baumannii* pneumonia, transposon-mediated disruption of OmpA decreased mortality in the experimental mice, suggesting a virulence function of the OmpA (Schweppe *et al.*, 2015).

The lipopolysaccharides (LPS) (endotoxin) has a major impact on *Acinetobacter* spp. virulence, inducing inflammatory cytokines such as interleukin-8 (IL-8) and tumour necrosis factor (TNF) from human monocytes equivalent to the levels of *Escherichia coli* stimulation. The stimulation was achieved via Toll-like receptor 4 (TLR-4) signalling (Erridge *et al.*, 2007a). It has also been shown that inhibition of LPS production via LpxC inhibitor (which affects lipid A biosynthesis) did not hinder the growth of the bacteria but reduce its virulence due to reduction of LPS-mediated activation of TLR-4, thus reducing inflammation and protecting the host from lethal infection (Lin *et al.*, 2012). However, another study had shown that mutation in lpxC clearly diminished the *in vitro* growth rate of the bacteria and attenuated its infection in the mouse models (Beceiro *et al.*, 2014).

1.4.2 Immunological Defence Mechanisms

The innate immune mechanism plays a vital role against *Acinetobacter* infection. A research on the immune response of mice intranasally inoculated with *A. baumannii* revealed that neutrophils were rapidly recruited to the lungs and declined to baseline levels upon clearance of the infection. Conversely, in a neutrophil-depleted

model of infection, there was enhancement of bacterial burden in the lungs with extrapulmonary bacterial dissemination (van Faassen *et al.*, 2007). Similar observation was made when nonlethal inoculum of clinical *A. baumannii* was administered intraperitoneally into mice that had been pre-treated with antineutrophil antibodies. The infection became rapidly lethal in the absence of neutrophils (Breslow *et al.*, 2011). These findings highlight the importance of neutrophils in early host defence against *A. baumannii*.

Macrophages also play an important part in the early, innate host defence. Macrophages efficiently phagocytosed and killed *A. baumannii* to limit their initial replication, released proinflammatory cytokines and recruited other cells of the innate immune system such as neutrophils (Qiu *et al.*, 2012). In another research, the loss of Fus1, a tumour suppressor protein with immunoregulatory function resulted in an earlier and more robust recruitment of neutrophils and macrophages in the lungs of mice infected with *A. baumannii* with concomitant increase in phagocytosis and clearance of the bacteria (Hood *et al.*, 2013). It can be concluded that neutrophils and macrophages are vital in the initial stages of the host immune defence to lower bacterial density, thus preventing the infection from becoming lethal.

TLR4 on immune cells had been implicated as the surface protein that was stimulated by *A. baumannii* endotoxin LPS. TLR4 gene-deficient mice was unable to mount the normal pulmonary chemokine/cytokine response post-intranasal infection, resulting in delayed onset of lung inflammation and increased bacterial count (Knapp *et al.*, 2006). Similar findings were demonstrated in mice injected with turpentine to suppress the chemokine/cytokine response. These intranasally infected mice also showed reduced clearance of *A. baumannii*, however they sustained less host damage due to the dampen inflammatory chemokine response. Thus, this suggest that host

outcome is not only dependent on bacterial density, but also the level of host inflammatory response towards *A. baumannii*.

The importance of interaction between host innate immune system and bacterial virulence in predicting outcome is further corroborated in a study by Bruhn *et al*. In this study, strains of differing virulence were evaluated in the intravenous infection model of immunocompetent mice (Bruhn *et al.*, 2015). Hypervirulent *A. baumannii* strains sustained high blood bacterial densities ($>10^7$ CFU/ml) at one-hour postinfection, which was maintained up to 24 hours. The hypervirulent strain was also better at evading complement-mediated killing and macrophage uptake compared to virulent and avirulent strains. The virulent strain achieved 1000-fold lower bacterial densities at one-hour postinfection and was only minimally cleared over the subsequent 23 hours. The avirulent strain had similar bacterial density to virulent strain at one-hour postinfection, and underwent an additional 100-fold decrease by 24-hour post infection due to its susceptibility to macrophage uptake and complement-mediated killing (Bruhn *et al.*, 2015).

A corresponding 10- to 100-fold increase of blood bacterial density of the avirulent strain can be seen when the mice were depleted of either macrophages, complement or neutrophils. Depletion of two of the immune effectors compounded the increase in blood bacterial density, while depletion of all three effectors synergistically increase the blood bacterial density to the level comparable to the hypervirulent strain. The blood bacterial densities of each strain at one-hour postinfection predicted lethality of infection (Bruhn *et al.*, 2015). Similar finding had been demonstrated in another study which use an *A. baumannii* murine wound model. In this study, neutrophil depletion lead to enhancement of bacterial burden, reduction of wound healing and

alteration of pro-inflammatory cytokine release resulting in severe microbial disease (Grguric-Smith *et al.*, 2015).

In summary, once inside the host, *A. baumannii* pathogenicity is initiated by its ability to escape complement and phagocytosis via its capsular composition. An early high load of infectious inoculum and reduction of host innate effectors, namely neutrophils and macrophages, also help the pathogen in escaping the host defence mechanism. Further damage to the host was attained by the pathogen's LPS triggering of TLR-4 mediated sepsis. The understanding of *Acinetobacter* spp. pathogenesis highlights the need for early effective therapy that could prevent high bacterial densities in blood and tissues, thus enabling the host to clear the pathogen rapidly and avoiding successive host damage from sepsis response.

1.5 Antibiotic Resistance

Antibiotic resistance is not a traditional virulence factor of *Acinetobacter* sp., however it is certainly the most important determinant of clinical outcome. Resistance is the main challenge of treating *Acinetobacter* infections since it limits the choice of antibiotics available to kill the infecting strain. Furthermore, *A. baumannii* is one of several Gram-negative species that commonly demonstrates an extensively drug resistance (XDR) phenotype, defined as *Acinetobacter* sp. isolate that is resistant to penicillins, cephalosporins, fluroquinolones and aminoglycosides (the multidrug resistant definition) and also resistant to carbapenems (Manchanda *et al.*, 2010). Infections by carbapenem-resistant strains had been shown to cause greater mortality and prolonged hospitalization compared to carbapenem-susceptible strains (Esterly *et al.*, 2011).

A. baumannii has a large resistance island within its genome, which contain 45 resistance genes (Adams *et al.*, 2008). Additionally, it can rapidly acquire other genetic entities for resistance from other bacterial species (Blackwell *et al.*, 2016) and develop antibiotic resistance in the middle of the course of therapy (Cheng *et al.*, 2015). The mechanisms of resistance of *A. baumannii* can be broadly categorized as enzymatic mechanisms; which is the most prevalent mechanism of β-lactam resistance in *A. baumannii*, and non-enzymatic mechanisms.

1.5.1 Enzymatic Mechanisms of Resistance

Enzymatic degradation by β-lactamases result in resistance towards β-lactams antibiotics. However, due to the complex nature of the pathogen, the interplay of multiple differing mechanisms may produce the same phenotype.

1.5.1.1 Cephalosporinases and Extended Spectrum β-Lactamases (ESBL)

All *A. baumannii* strains are inherently encoded with AmpC cephalosporinases gene in their chromosomes, also known as *Acinetobacter*-derived cephalosporinases (Hujer *et al.*, 2005). Overexpression of this enzyme in *A. baumannii* is determined by the presence of an upstream insertion sequence (IS) element known as IS*Aba1*. Presence of this element highly correlates with augmented AmpC gene expression and resistant to extended-spectrum cephalosporin (Corvec *et al.*, 2003). Nevertheless, cefepime and carbapenems is stable in response to these enzymes. ESBL from the Ambler class A group have also been detected in *A. baumannii*. The ESBL that had been implicated include VEB-1, PER-1 and -2 (Farajnia *et al.*, 2013), TEM-92 and -116, SHV-12 (Naiemi *et al.*, 2005) and CTX-M-2 and -43 (Celenza *et al.*, 2006).

1.5.1.2 Oxacillinases

Carbapenemases which are present in *A. baumannii* include the serine oxacillinases (Ambler class D OXA type) and the metallo-β-lactamases (Ambler class B). The first identified OXA-type enzyme with carbapenem-hydrolyzing activity was detected from a clinical strain in 1985 and was initially named ARI-1 (Paton *et al.*, 1993). The enzyme was derived from a plasmid-based resistance gene which was found to be transferable and conferred resistant towards imipenem. The gene was later sequenced and named *bla*$_{\text{OXA-23}}$ (Donald *et al.*, 2000). OXA-27 and OXA-49 are two other closely related enzymes that make up the *bla*$_{\text{OXA-23}}$ gene cluster in *A. baumannii*. Subsequently, other OXA-type gene clusters with carbapenemases activity have been described, including the *bla*$_{\text{OXA-24}}$-like (can be chromosomal or plasmid based) which encodes for OXA-24, -25, -26 and -40, and plasmid-based *bla*$_{\text{OXA-58}}$-like which encodes for OXA-58. The final gene cluster and the most prevalent is the *bla*$_{\text{OXA-51}}$-like which encodes for OXA-51, -64, -65, -66, -68, -69, -70, -71, -78, -79, -80 and -82. The gene cluster is intrinsic to *A. baumannii* and chromosomally based.

The presence of the plasmid-based oxacillinases had been shown to contribute significantly to the carbapenem-hydrolysing capacity of *A. baumannii* (Héritier *et al.*, 2005). Similar to the ADC, expression of these OXA-type gene clusters in *A. baumannii* is dependent on the presence of insertion sequences such as IS*Aba1* and IS*Aba3* laying upstream to the gene clusters. For the chromosomally-encoded *bla*$_{\text{OXA-51}}$-like gene, only isolates with IS*Aba1* adjacent to the *bla*$_{\text{OXA-51}}$-like gene were carbapenem resistance (Turton *et al.*, 2006a). The IS elements are mobile since they encode a transposase and they also contain promoter regions that lead to overexpression of downstream resistance genes.

Surveillance study of *A. baumannii* strains from the Asian regions showed that the *bla*$_{\text{OXA-51}}$-like were detected in all strains. Among the plasmid-mediated

oxacillinases, *bla*$_{\text{OXA-23}}$-like was the most prevalent and had been detected from almost all Asian countries (Kim *et al.*, 2013). The study also found *bla*$_{\text{OXA-24}}$-like in Taiwan while *bla*$_{\text{OXA-58}}$-like was not found in any of the strains. Worldwide, *A. baumannii* strains containing *bla*$_{\text{OXA-24}}$-like had been discovered in Spain, Belgium, China, South Korea and Bahrain (Poirel *et al.*, 2010) while *bla*$_{\text{OXA-58}}$-like had been detected in strains from France, United Kingdom, Argentina and Kuwait (Coelho *et al.*, 2006).

1.5.1.3 Metallo-β-lactamases (MBLs)

A. baumannii also possesses MBLs (Ambler class B). Even though they are less prevalent compared to serine oxacillinases in the organism, these MBLs have a more potent hydrolytic activity (100 – 1000-fold) towards carbapenems compared to the OXA-type carbapenemases (Poirel and Nordmann, 2006). MBLs can hydrolyse all β-lactam except aztreonam. All the MBLs (IMP, VIM, SIM and NDM) had been found in *Acinetobacter* sp. The *bla*$_{\text{NDM}}$ gene is usually found flanked by two copies of the IS*Aba125* element, forming a composite transposon named Tn*125* (Bonnin *et al.*, 2012). The discovery of a truncated form of this composite transposon in *Enterobacteriaceae* in contrast to its entire form in *A .baumannii* is strongly suggestive of the role *Acinetobacter* plays in spreading *bla*$_{\text{NDM}}$ genes from its natural reservoir to *Enterobactericeae* (Bonnin et al., 2014).

Table 1.1 Summary of β-lactamases presents in *A. baumannii* (based on Ambler classification)

Ambler Class	**Active Site**	**Enzyme Type**	**Subtrates**	**Enzymes found in *A. baumannii***
A	Serine	Extended-spectrum (β-lactamase)	Benzylpenicillin, aminopenicillins, carboxypenicillins, ureidopenicillins, narrow-spectrum cephalosporins, oxymino-β-lactams (cefotaxime, ceftazidime, ceftriaxone) and aztreonam	VEB-1, PER-1 and -2, TEM-92 and -116, SHV-12 and CTX-M-2 and -43
B	Metallo-β-lactamases	Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	IMP, VIM, SIM and NDM
C	Serine	Cephalosporinases	Substrates of extended-spectrum cephamycins,	AmpC-type enzymes
D	Serine	Carbapenem-hydrolyzing oxacillinases	Substrates of extended-spectrum plus cephamycins and carbapenems	OXA-23 until -27, OXA-40, OXA-49, OXA-51, OXA-58, OXA-64 until -82,

AmpC, ampicillin C; CTX-M-2 and -23, cefotaxime-M-2 and -23; IMP, imipenem; NDM-1, New Delhi metallo-β-lactamase-1; OXA, oxacillin; PER-1, Pseudomonas extended resistance-1; SHV-12, sulfhydryl variable-12; SIM, Seoul imipenemase; TEM-92 and -116, Temoneira-92 and -116; VIM, Verona integron-encoded metallo-β-lactamase.

1.5.2 Non-enzymatic Mechanisms of Resistance

Nonenzymatic mechanisms also had a function in conferring resistance against carbapenem and other antibiotics in *A. baumannii*. These changes include changes in outer membrane proteins (OMPs), multidrug efflux pumps and alterations in the affinity or expression of specific antibiotic binding sites. As compared to other gram-negative

organisms, *A. baumannii* generally had low permeability to antibiotic due to the small number and size of porins in its outer membrane. Two of these major porins has been implicated in carbapenem resistance, namely CarO and OprD (Dupont *et al.*, 2005; Siroy *et al.*, 2005). These porins had been shown to allow influx of carbapenem and the reduction in *carO* transcription lead to downregulation of the CarO porin system thus a decrease in carbapenem influx.

Multidrug efflux systems is also an important mechanism of resistance in *A. baumannii*, the most prominent being the resistance-nodulation-division (RND) family-type pump AdeABC. The AdeABC pump substrate profile includes β-lactams, aminoglycosides, erythromycin, chloramphenicol, tetracyclines, fluoroquinolones, trimethoprim and ethidium bromide (Nemec *et al.*, 2007). AdeABC is chromosomally encoded and has a three-component structure: AdeB forms the transmembrane component, AdeA forms the inner membrane fusion protein and AdeC forms the OMP. It is regulated by a two-component system with a sensor kinase (AdeS) and its associated response regulator (AdeR) (Marchand *et al.*, 2004).

A. baumannii also contains genes that codes for resistance determinants specific to certain antibiotics. One of them is the aminoglycoside-modifying enzymes which structurally modify aminoglycosides, rendering them inactive (Moniri *et al.*, 2010). The 16S rRNA methylase ArmA which impairs aminoglycoside binding to its target site and confers high-level resistance to all aminoglycosides had also been detected in *A. baumannii* (Doi *et al.*, 2007). Other than that, mutations in the *gyrA* and *parC* genes had been described. These mutations result in modifications to DNA gyrase or topoisomerase IV which subsequently interfere with target site binding, thus conferring resistance to quinolones (Higgins *et al.*, 2004). In the case of tetracycline resistance, the

tet(M) gene which confers ribosomal protection had been detected in *A. baumannii* (Ribera *et al.*, 2003).

Resistance against polymyxin, the last resort antimicrobial agent against XDR *A. baumannii*, was shown to be mediated by the complete loss of the initial binding target, the lipid A component of LPS (Moffatt *et al.*, 2010). This is due to the mutation of one of the first three genes of the lipid A biosynthesis pathway; *lpxA, lpxC* or *lpxD*. The LPS-deficient colistin-resistant strain had a less negative charge thus reduced affinity towards colistin. Another possible mechanism of polymyxin resistance is through addition of phosphoethanolamine to hepta-acylated lipid A (Beceiro *et al.*, 2011). This was a result of one amino acid change in PmrB and up-regulated expression of *pmrA* and *pmrB*. The LPS modification also lead to colistin resistance.

1.6 Clinical Manifestation

1.6.1 Risk Factors for Infection

The ability of *Acinetobacter* spp. to persist on environmental surfaces and colonize the hands of healthcare workers transiently partly explains why infections is highly likely among hospitalized patients with disrupted anatomical barrier i.e. mechanical ventilation, urinary catheterization etc. Longer duration of hospital stay until infection, ICU stay, emergent surgical operation, total parenteral nutrition and having a central venous catheter, endotracheal tube, urinary catheter or nasogastric tube had all been associated with increased risk for MDR *A. baumannii* infection (Baran *et al.*, 2008).

Other risk factors for infection include prior use of certain antibiotics, especially the use of carbapenem (Corbella *et al.*, 2000). Exposure to carbapenem gives a selective advantage for carbapenem-resistance strains over carbapenem-susceptible *A. baumannii*

strains. Other antibiotics that had been implicated include third-generation cephalosporins; a case-control study had shown that the use of third generation cephalosporins increase the risk of *A. baumannii*, regardless of susceptibility towards imipenem (Lee *et al.*, 2004).

Colonization by MDR *A. baumannii* is also an important predisposing factor for subsequent infection. A study had revealed that 41% of the patient that underwent rectal swab screening in ICU was colonized by *A. baumannii*, with 71% of them acquiring the organism within their first week of ICU stay (Corbella *et al.*, 1996). Clinical infections occurred more frequently among those who are colonized compared to those who are not.

Even though it is widely accepted that *Acinetobacter* sp. cause infections mainly in the immunocompromised patients, patients with neutropenia constitutes only a minority of those infected (Freire *et al.*, 2016). Hence, focus should be on avoiding unnecessary invasive procedures and antimicrobial stewardship to reduce the risk for infection with this highly resistant pathogen.

1.6.2 Types of Infection

1.6.2.1 Ventricular-associated Pneumonia (VAP)

A. baumannii is an established hospital pathogen, and its most common clinical manifestations are hospital-acquired pneumonia and bacteraemia. The pathogen can adhere to plastic and form biofilms on endotracheal tube, which would become an ideal nidus for its environmental transmission (Gil-Perotin *et al.*, 2012). Intubated patient would become infected through aspiration. Since natural host barriers such as the cough reflex is compromised in mechanically ventilated patient, aspirated droplets of the pathogen can go directly to the alveoli and establish infection in the tissue.

Acinetobacter spp. accounted for 6.1% of VAP cases in the United States from 2011 to 2012, with 55.5% to 63.5% of the tested isolates showing carbapenem resistance (Weiner *et al.*, 2016). The rates of *A. baumannii* VAP were significantly higher in the South East Asian region; this was demonstrated by a study in Thailand whereby *A. baumannii* accounted for 54.3% of VAP cases with 90.2% of them being drug-resistant strains (Inchai *et al.*, 2015b). It had been shown that among (VAP) patients, those infected with imipenem-resistance *A. baumannii* had longer hospital stay before VAP onset and higher hospital mortality rates compared to those with imipenem-susceptible isolates (Chang *et al.*, 2011).

1.6.2.2 Blood-stream Infection

Likewise, *Acinetobacter* sp. bacteraemia usually occurs in the presence of intravascular catheters and could also be secondary to pneumonia (Wisplinghoff *et al.*, 2012). From 2011 to 2014, *A. baumannii* represented 1.4% of the total isolates responsible for central line-associated bloodstream infection in the United States, with carbapenem-resistance rates ranging from 46.6% to 57.2% of the tested *Acinetobacter* isolates. Data from Europe showed that 2.5% of ICU-acquired bloodstream infections are caused by *Acinetobacter* sp., with carbapenem-resistant rates of 63.5% (ECDC, 2016). A local study had shown that the attack rate of *Acinetobacter* sp. blood stream infection was 2.77 episodes per 1000 hospital admissions (Deris *et al.*, 2009). In term of mortality, one study found that there was no statistically significant difference in term of mortality caused by MDR *A. baumannii* or non-MDR *A. baumannii* bacteraemia (Chopra *et al.*, 2013).

1.6.2.3 Other Nosocomial Infections

Acinetobacter sp. accounts for 0.7% of catheter-associated urinary tract infections (CAUTI) in the United States from 2011 to 2014, with carbapenem-resistance rates 57.7% to 69% (Weiner *et al.*, 2016). Other recognised clinical manifestations include meningitis (usually in the postoperative settings) (Wroblewska *et al.*, 2004) and osteomyelitis. One study had shown that *A. baumannii* accounts for 21% of cases of gram-negative osteomyelitis, with 40% of them being resistant to carbapenem (Carvalho *et al.*, 2012).

1.6.2.4 Community-acquired Infections

Apart from nosocomial infections, community-acquired infections of *Acinetobacter* sp. had been reported especially from countries with tropical and subtropical climate. Most of these infections are pneumonia, and patients affected usually had underlying comorbidities such as diabetes, chronic obstructive pulmonary disease, renal disease and lifestyle risks such as heavy smoking and excessive alcohol consumption (Falagas *et al.*, 2007). *A. baumannii* with multiple drug resistance had also been implicated in cases of blood stream infections among injured US military personnels in Iraq and Afghanistan (Center for Disease Control and Prevention, 2004).

1.6.3 Outcomes of XDR *A. baumannii* Infection

XDR *A. baumannii* infection has been associated with greater mortality, increased costs and longer hospitalization. A study in Taiwan found that patients with *A. baumannii* complex bacteraemia had 33.6% mortality rate, and those infected who are infected with the imipenem resistant strains having significantly higher mortality at 70% (Lee *et al.*, 2014b). Another study revealed that imipenem resistance *A. baumannii* cases had prolonged duration of bacteraemia compared to those infected with imipenem susceptible strains (Deris *et al.*, 2011). A systematic review also showed that patient

with carbapenem-resistant *A. baumannii* had a significantly higher risk of mortality than patients with carbapenem-susceptible *A. baumannii*, but it was also noted that the carbapenem-resistant group had more severe underlying medical illness and more likely to receive inappropriate empirical therapy (Lemos *et al.*, 2014). Patients with imipenem-resistant *A. baumannii* isolates, regardless of colonization or infection, also had greater length of hospital stay and greater hospital charges after culture (Lautenbach *et al.*, 2015).

1.7 Laboratory Identification

1.7.1 Microscopy and Culture

Acinetobacter sp. appears as gram negative coccobacillary cells commonly diplococci under Gram staining. *Acinetobacter* sp. can be difficult to destain, thus maybe misidentified as either gram-negative or gram-positive cocci in direct smears of clinical specimens (Das *et al.*, 1997). The organism grows well on solid media that are commonly used in clinical microbiology laboratories, such as sheep blood agar & tryptic soy agar at a 37 °C incubation temperature. After 24 hours incubation, the colonies are between 0.5 and 2 mm in diameter, translucent to opaque (non-pigmented), convex and entire. Most strains grow well on MacConkey agar and produce a faint pink tint. Some *Acinetobacter* sp. outside the *A. baumannii* complex may not grow on MacConkey agar.

1.7.2 Presumptive Identification via Phenotypical Characteristic

Presumptive identification of *Acinetobacter* sp. to the genus level can be made based on the lack of cytochrome oxidase activity, lack of motility and resistance to penicillin. Inoculation on the triple sugar ion agar usually yield an alkaline slant and a neutral butt, indicating that the bacteria does not utilize glucose and lactose or sucrose.

Bouvet et al conducted a study to identify isolates based on the first twelve discovered *Acinetobacter* genospecies delineated by DNA-DNA hybridization through phenotypic properties (Bouvet and Grimont, 1987). The strains were identified based on growth at 37 °C, 41 °C and 44 °C, production of acid from glucose, gelatin hydrolysis and fourteen carbon sources utilization tests. However, a subsequent study using similar methods revealed problems differentiating *A. calcoaceticus, A. baumannii, A. pittii,* and *A. nosocomialis* phenotypically, thus it has been proposed to refer to these species as the *A. calcoaceticus-A. baumannii* complex (Gerner-Smidt *et al.*, 1991).

Even manual identification system such as the API 20NE was unable to correctly distinguish between *A. calcoaceticus, A. baumannii*, *A. pittii*, and *A. nosocomialis*, and it was suggested that these species were grouped together in *A. calcoaceticus-A. baumannii* complex to improve the database accuracy (Bernards *et al.*, 1996) The use of semi-automated system such as Vitek 2 also showed that only 76% of *A. baumannii* can be correctly identified by the system (Joyanes *et al.*, 2001).

1.7.3 Identification via Genotypic Methods

Several genotypic methods have been developed to identify *Acinetobacter* sp. Methods include amplified ribosomal DNA restriction analysis (ANDRA) (Vaneechoutte *et al.*, 1995), partial rpoB gene sequence analysis (Gundi *et al.*, 2009) and Fourier transform infrared spectroscopy (FITR) (Sousa *et al.*, 2014). Amplification and sequencing of species-specific DNA regions such as intrinsic oxacillinases i.e $bla_{\text{OXA-51}}$-like for *A. baumannii* was also widely used (Turton *et al.*, 2006b).

Currently, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) seems to be the most practical method for routine use in clinical microbiology laboratory to accurately identify *A. baumannii.* When specific

signature profiles for *A. baumannii*, *A. nosocomialis* and *A. pittii* was included in the Bruker database, MALDI-TOF MS were able to correctly differentiate between these three species within the *A. baumannii* complex (Espinal *et al.*, 2012). *A. seifertii* and *A. dijkshoorniae*, the two newest member of the *A. baumannii* complex, had also been successfully identified using MALDI-TOF MS with a positive predictive value of 99.6% (777/780) and 96.8% (302/312), respectively (Mari-Almirall *et al.*, 2017).

1.7.4 Antimicrobial Sensitivity Testing

Acinetobacter sp. had been shown to be universally resistant to penicillin, ampicillin, and oxyimino-cephalosporinases due to the presence of intrinsic chromosomal AmpC betalactamase. Most strains are also resistant to chloramphenicol. *A. baumannii* is generally more resistant to antimicrobial as compared to non-*A. baumannii* strains (Seifert *et al.*, 1993).

Broth microdilution or disk diffusion methods can be performed for susceptibility testing of *Acinetobacter* sp. However, broth microdilution test especially for beta-lactams exhibited subtle growth patterns that were difficult to interpret, leading to major discrepancies between microdilution and disk diffusion susceptibility results (Swenson *et al.*, 2004). On the other hand, tigecycline minimum inhibitory concentration (MIC) was falsely high when tested via Etest compared to broth microdilution method (Swenson *et al.*, 2004). Susceptibility breakpoints has not been published by either Clinical Laboratory Standard Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) for tigecycline since the antibiotic was not indicated for *Acinetobacter* infections. Nonetheless, tigecycline has been used for treatment of *Acinetobacter* infections in the wake of increasing antimicrobial resistance and limited therapeutic options. Based on multicentre studies,

Jones et al had proposed that disc diffusion zone diameter of more or equal to 16 mm be regarded as sensitive while zone size less or equal to 12 mm be regarded as resistant to tigecycline (Jones *et al.*, 2007).

Previously, there were different breakpoints for many of the antibiotics between CLSI and EUCAST. However, the considerable differences have been eliminated when CLSI lowered its MIC breakpoints for *A. baumannii* to become very similar or identical to those used by EUCAST. Until now, no methods to detect the presence of the widely prevalent OXA-type carbapenemase in *A. baumannii* has been validated by CLSI. Detection of the OXA-type enzymes mainly depends on molecular detection of the bla_{OXA} genes (Woodford *et al.*, 2006).

1.8 Treatment of XDR *A. baumannii*

1.8.1 Single Antimicrobial Therapy

Due to the limited therapeutic options available for the treatment of XDR *A. baumannii*, there is currently no consensus on optimal antimicrobial treatment of such strains. Antimicrobial regime can be administered as monotherapy or combination therapy; the latter being preferred to prevent new emergence of resistance and improve treatment outcome.

One of the most preferred agent for monotherapy treatment of XDR *A. baumannii* is polymyxin. Polymyxins are polycationic lipopeptide that act both on the outer and cytoplasmic membranes of bacteria leading to loss of membrane integrity. Polymyxin B and polymyxin E (colistin) are suitable for clinical use. The antibiotic had been around for around 50 years, but its usage had been revived in the last decade in the face of emerging multidrug resistant gram negative bacteria. In a retrospective cohort study, colistin therapy for multidrug resistant (MDR) *A. baumannii* infection

successfully cured 138 (81.2%) of the patients, with 20 of them receiving monotherapy (Falagas *et al.*, 2010). 75% cure rate had also been achieved among patients treated with colistin for VAP caused by *A. baumannii* and *P. aeruginosa* susceptible only to colistin (Neonakis *et al.*).

An inadequacy of polymyxin, especially for treatment of *Acinetobacter* pneumonia, is that it has relatively poor penetration in the lungs. Intravenous administration of colistin 2 million international units three times daily in critically ill patients results in suboptimal serum concentration and were undetectable in the bronchoalveolar lavage of these patients (Imberti *et al.*, 2010). The use of inhaled colistin methanesulfonate was associated with eradication of MDR *A. baumannii* within 14 days after the index day and shortened the duration of MDR *A. baumannii* recovery from the respiratory tract by 2 weeks (Kuo *et al.*, 2012). Nebulized polymyxins could achieve very high concentrations in the lungs with reduced systemic exposure and risk of toxicity. However, another study had discovered that there were no significant differences in terms of clinical success, clinical failure and recurrence between patients receiving parenteral colistin only and those receiving parenteral and nebulized colistin (Demirdal *et al.*, 2016).

Unfortunately, resistance had been reported against polymyxin, albeit the rates were low. Polymyxin B resistance rates was 2.1% among *Acinetobacter* spp., however the rates were slightly higher among carbapenem-resistance (2.8%) and multidrug resistance strains (3.2%) (Gales *et al.*, 2006).

Another agent commonly used as monotherapy is sulbactam. Sulbactam is a beta-lactamase inhibitor for most bacteria, however it has direct antimicrobial activity against *A. baumannii*. The activity of sulbactam against *A. baumannii* is mediated by

inhibition of penicillin-binding proteins (PBP) PBP1 and PBP3 (Penwell *et al.*, 2015). A rare *pbp3* mutants which confer high level resistant against sulbactam has also been discovered but may cost attenuation in fitness (Penwell *et al.*, 2015). Unfortunately, resistance against sulbactam had been reported; in Taiwan the resistance rate was 70% (Yang *et al.*, 2010) and in Spain only 46.7% of the *A. baumannii* isolates were susceptible to sulbactam (Fernández-Cuenca *et al.*, 2004). It is worth noting that in many countries, sulbactam is commercially available only in fixed dose combination with either ampicillin or cefoperazone.

Tigecycline had also been used as an option for treatment of XDR *A. baumannii*. It is the first of a novel class of minocycline derivatives known as the glycylcyclines. The drug binds with high affinity to bacterial ribosomes and is unaffected by tetracycline (*tet)* efflux and ribosomal protection systems. However, it serum concentration is low and its use for treatment had been associated with increased mortality and non-cure rates (Prasad *et al.*, 2012). Specifically, MDR *Acinetobacter* bacteraemia may have inferior outcomes when treated with tigecycline including worse survival and failure to clear bacteraemia, even if the MIC $\leq$ 2 μg/ml (Gordon and Wareham, 2009).

As an alternative to tigecycline, minocycline has been considered as a therapeutic agent against MDR *Acinetobacter* infections. A global surveillance program including XDR *Acinetobacter* sp. strains revealed that minocycline was the most active tetracycline in vitro (according to the CLSI breakpoint of MIC $\leq$ 4 μg/ml) (Flamm *et al.*, 2016). A case series of 55 patients treated with minocycline for MDR *A. baumannii* showed that more than 70% had favourable clinical response, however only three received monotherapies (Goff *et al.*, 2014).