ANTIMICROBIAL SUSCEPTIBILITY AND MOLECULAR PROFILES OF *ACINETOBACTER BAUMANNII* IN MAKKAH HOSPITALS, AND THE POTENTIAL USE OF BACTERIOPHAGE AS A TREATMENT OPTION

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LIST OF ABBREVIATIONS

AbOmpA	outer membrane protein A
Acb	*Acinetobacter baumannii* complex
AMR	antimicrobial resistance
ATM	amplicon tagment mix
CDC	Center for Disease Control
CLSI	Clinical and Laboratory Standards Institute
CPS	capsular polysaccharides
CRAB	carbapemen-resistant *A. baumannii*
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
ICU	intensive care unit
kbp	kilo base pair
LPS	lipopolysaccharide
MDR	multi drug resistant
MLST	multi locus sequence typing
NGS	next generation sequencing
NT	neutralize tagment buffer
OMV	outer membrane vesicles
OmpA	outer membrane vesicle protein A
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PHASTER	PHAge Search Tool Enhanced Release
PLD	phospholipase D
SAMSO	Saudi Aramco Medical Services Organization
SNP	single nucleotide polymorphism
spp	species

SRST2	short read sequence typing for bacterial pathogens
ST	sequence type
VF	virulence factors
WHO	World Health Organization

KERENTANAN ANTIMIKROB DAN PROFIL MOLEKUL *ACINETOBACTER BAUMANNII* DI HOSPITAL-HOSPITAL DI MAKKAH, SERTA POTENSI PENGGUNAAN BAKTERIOFAJ SEBAGAI PILIHAN RAWATAN

ABSTRAK

Acinetobacter baumannii yang merupakan bakteria Gram-negatif yang kerapkai menyebabkan jangkitan terutamanya di unit rawatan rapi seluruh dunia termasuk di Arab Saudi. Kuman ini boleh mengalami mutasi menyebabkan rintang terhadap hampir semua agen antimikrob yang sedia ada. Untuk memahami magnitud masalah *A. baumannii* setempat, 895 pencilan dikumpulkan dari King Abdullah Medical City Makkah, Hospital Al Noor dan Hospital Al Zahir Makkah dari tahun 2013-2019. Sistem automatik oleh Vitek2® digunakan untuk mengenal pasti organisma dan juga digunakan untuk ujian kerentanan antimikrob. Tiga puluh pencilan MDR *A. baumannii* dari King Abdullah Medical City Makkah dan tujuh dari Hospital Al Noor pemprofilan telah dipilih untuk analisis keseluruhan penjujukan genom. Untuk mengkaji peranan bakteriofaj, sampel air dari kumbahan King Abdullah Medical City Makkah dan Hospital Al Noor telah disaring kehadiran bakteriofaj. Beberapa calon bakteriofaj ditemui, tetapi ujian litik berikutnya adalah negatif. Fenomena ini dikaji dengan menyemak kehadiran genom bakteriofaj yang masuk dalam asid nukleik bakteria menjadi profaj. Dalam kajian ini, 70-80% daripada *A. baumannii* yang dipencilkan dari hospital-hospital di Makkah didapati rintang terhadap antibiotik yang biasa digunakan di unit rawatan rapi. Terdapat kecenderungan peningkatan rintangan terhadap agen yang dikhaskan untuk merawat *A. baumannii* yang rintang terhadap carbapenem iaitu tigecycline dan colistin. ST-195 adalah jenis jujukan utama, menyumbang kepada 48.6% jangkitan *A. baumannii* di hospital Makkah. Terdapat ST yang baru dikenalpasti yang dikaitkan dengan 18.9% jangkitan memerlukan pencirian

lanjut. Kajian ini mendapati *bla*$_{\text{ADC-25}}$ dan *bla*$_{\text{OXA-66}}$ adalah yang paling biasa menyebabkan bakteria rintang terhadap antibiotik β-laktam, dengan masing-masing 86.5% dan 83.8%, diikuti dengan *bla*$_{\text{OXA-23}}$ dan *bla* $_{\text{TEM-1D}}$ masing-masing pada 37.8% dan 37.8%. Kajian ini juga mendapati 75.7% dan 73.0% daripada MDR *A. baumannii* yang dipencilkan dari hospital Makkah memperolehi gen rintang makrolida *mph(E)* dan *msr(E)*. Rintangan terhadap aminoglycosides dikodkan terutamanya oleh gen aminoglycoside phosphotransferase, *aph*(3")-Ib pada 83.8% dan aminoglycoside O-phosphotransferase *aph(6)-Id* pada 70.3%. Selain itu, kuman MDR ini juga diperoleh daripada gen tahan sulfonamida *sul1* (32.4%) dan *sul2* (18.9%). Teras projek ini adalah untuk mencari virus bakteriofaj yang mempunyai keupayaan untuk menjangkiti dan membunuhkan sel *A. baumannii*. Selepas mencari bakteriofaj secara meluas daripada air kumbahan dua hospital di Makkah, beberapa calon bacteriofaj telah disenarai pendek. Namun bakteriofaj ini gagal memberikan keputusan secara konsisten dalam kajian *in vitro*. Kami dapati hampir satu perempat daripada pencilan *A. baumannii* dari hospital di Makkah mempunyai gen bacteriofaj yang lengkap. Dua pencilan iaitu AB417 dan AB552 telah dicirikan lanjut, sebelum dan selepas ujian pendedahan terhadap bacteriofaj. Terdapat tambahan jujukan lengkap bakteriofaj dalam pencilan AB552 dan untuk kali pertama, tiga genom non-*Acinetobacter* bakteriofaj di dalam jujukan MDR *Acinetobacter* telah dijumpai. Kesimpulannya, kajian ini mendapati kadar ketahanan *A. baumannii* terhadap agen antimikrob biasa adalah lebih daripada 80% di hospital Makkah, yang boleh menyebabkan kegagalan rawatan jika antibiotik ini digunakan untuk merawat jangkitan. Pencarian pendekatan bukan farmakologi dengan menggunakan terapi bacteriofaj menunjukkan hasil yang tidak konsisten. Dengan pilihan rawatan yang terhad, langkah kawalan infeksi yang berkesan dan program pengawasan antibiotik adalah elemen utama untuk

membendung jenis bakteria rintang ini daripada tersebar. Kajian lanjut adalah sangat penting untuk mencari agen alternatif untuk merawat MDR *A. baumannii*.

ANTIMICROBIAL SUSCEPTIBILITY AND MOLECULAR PROFILES OF *ACINETOBACTER BAUMANNII* IN MAKKAH HOSPITALS, AND THE POTENTIAL USE OF BACTERIOPHAGE AS A TREATMENT OPTION

ABSTRACT

Acinetobacter baumannii is an opportunistic Gram-negative pathogen that frequently causes infections especially in intensive care settings worldwide including Saudi Arabia. This organism is known to acquire resistant to almost all clinically available antimicrobial agents. To understand the magnitude of *A. baumannii* acquisition in local settings, 895 isolates were collected from King Abdullah Medical City Makkah, Al Noor Hospital and Al Zahir Hospital of Makkah from 2013-2019. Vitek2® system was used for identification of the organism and antimicrobial susceptibility test. Thirty MDR *A. baumannii* isolates from King Abdullah Medical City Makkah and seven from Al Noor Hospital were selected for whole genome sequencing. To study the role of bacteriophage, sewage water from King Abdullah Medical City Makkah and Al Noor Hospital were screened for clinical isolates *A. baumannii* lytic phenomenon. A few bacteriophage candidates were found, but the subsequent lytic tests were negative. This phenomenon was studied by reviewing the bacteriophage genomes integrated in the bacterial nucleic acids. In this study, 70-80% of *A. baumannii* isolated from Makkah hospitals were found to be resistant to commonly used antibiotics in intensive care units. There are increasing trends of resistance to agents that been reserved to treat carbapenem-resistant *A. baumannii* i.e. tigecycline and colistin. The sequence type (ST)-195 was the predominant sequence type, contributed to 48.6% of *A. baumannii* isolations in Makkah hospitals. There were three novel sequence types that associated with 18.9% of infections that need further characterization. Among the β-lactamase resistant mutations, this study found

bla$_{\text{ADC-25}}$ and *bla*$_{\text{OXA-66}}$ were the most common with 86.5% and 83.8% respectively, followed by *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{TEM-1D}}$, both at 37.8%. This study also found 75.7% and 73.0% of the tested MDR *A. baumannii* isolated from Makkah hospitals acquired *mph*(E) and *msr*(E) macrolides resistant genes respectively. The aminoglycosides resistance was encoded mainly by aminoglycoside phosphor-transferase gene, *aph(3'')-Ib* at 83.8% and aminoglycoside O-phosphotransferase *aph(6)-Id* at 70.3%. Besides, these MDR isolates were also acquired of sulphonamide resistant genes of *sul*1 (32.4%) and *sul*2 (18.9%). Part of the core of this project was to find the potential bacteriophage that has capability to infect and lyse *A. baumannii* cells. After extensive searching for bacteriophage from sewage water of two tertiary care hospitals in Makkah, several bacteriophage candidates were shortlisted, however these bacteriophages failed to perform lytic phenomenon consistently. Almost one-fourth of MDR *A. baumannii* were found to acquire intact bacteriophage genomes, indicated prophages condition. The whole genome sequence of two MDR *A. baumannii* isolates (AB417 and AB552) were studied before and after bacteriophage treatment indicated additional intact bacteriophage genomes were added in isolate AB552. Three genomes of non-*Acinetobacter* bacteriophages was found to be integrated in these MDR *Acinetobacter* series. In conclusion, this study found the resistant rate of *A. baumannii* were more than 80% in Makkah hospitals which may lead to treatment failure in clinical practice. Searching for a non-pharmacological approach by means of using bacteriophage therapy showed inconsistent outcomes. With few treatment options available, robust infection control strategies and antibiotic stewardship programs are critical for preventing the spread of these resistant strains. Further research is very critical to find alternative agents to treat MDR *A. baumannii.*

CHAPTER 1

INTRODUCTION AND RATIONALE OF STUDY

1.1 Background of the study

Acinetobacter baumannii is a Gram-negative bacillus. It is responsible for a large number of nosocomial infections, most commonly in patients requiring critical care treatment. It is innately resistant to a wide variety of antibiotics and is capable of acquiring resistance to a variety of additional antibiotics via several methods. It survives on dry surfaces for months, which is responsible for its persistence in hospital environments and transmission.

With the introduction in the Kingdom of Saudi Arabia of carbapenem-resistant *A. baumannii* (CRAB), with limited treatment options. In some occasions, the only available drug seem to be polymyxins in synergy with other antibiotics, with few, if any, new agents in the pipeline. Studies on the molecular characterization and antibiotic profile of *A. baumannii* in Saudi Arabia (Figure 1.1) are still sparse.

Figure 1.1 In the Kingdom of Saudi Arabia, there are fourteen administrative regions. Makkah city located in Makkah Province at western region of the kingdom whereas Madinah is located north to Makkah is also attraction place to visit during hajj and umrah.

Makkah and Madinah (Figure 1.1) in particular, each year, millions of people from over 180 nations come to perform the hajj and umrah pilgrimage. This serves as a global exchange point for microorganisms and genetic material between diseases. Understanding the molecular epidemiology and resistance mutations of multidrug resistant organisms in this region is of considerable interest.

There are more than ten hospitals in Makkah city. These include King Abdullah Medical City Specialist Hospital, Al Noor Specialist Hospital, Ajyaad General Hospital and King Faisal Hospital. Beside these hospitals, every country's hajj missions develop temporary hospitals during hajj season to cater their own pilgrimages. It is expected that these hospitals can be the source of *A. baumannii* transmission.

With increasing multidrug resistant *A. baumannii*, the non-antimicrobial therapy needs to be sought out either as a complimentary or an additional mode of treatment, together with currently available antibiotics. Bacteriophage therapy is one of them. Bacteriophages are a kind of viruses that have capability to infect bacteria, lyse it and thus, kill the bacterial host. It was discovered in early last century, but the therapeutic use still under investigated. In early development, bacteriophages were used to treat dysentery, staphylococcal and streptococcal sepsis and typhoid fever. With the discovery of antibiotics, bacteriophage became unpopular. However, looking to emergence of bacteria that are resistant to clinically available antibiotics, the role of bacteriophage needs to be re-discovered.

1.2 Rationale of the Study

A. baumannii has become a significant nosocomial infection in recent years, notably in intensive care units (ICU). It has also been associated with multi drug resistance (MDR) and has been a real source of concern for the clinician in terms of decreasing and sometimes no antimicrobial treatment options. The aim of this study is to understand the common strains and mutations among *A. baumannii* isolates and the potential role of bacteriophage therapy for treating infections and controlling colonization with *A. baumannii*.

Currently, phage therapy is being used actively for the treatment of various multi drug resistant organisms. Thus, it has potential to be used as an alternative or as a conjunct treatment option for *A. baumannii* infections or colonizations. Bacteriophages are highly specific natural predators of bacteria and pose no threat to humans. Bacteriophages can be

isolated from the environment such as sewage water and then can be characterized for their selective infectivity against *A. baumannii.*

1.3 Research Objectives

1.3.1 General objective

To determine the susceptibility, clonality and resistant genes of *A. baumannii* from Makkah hospitals and to investigate the use of lytic bacteriophage as a treatment option for *A. baumannii*.

1.3.2 Specific Objective

- To determine the susceptibility patterns and clonality of *A. baumannii* in Makkah hospitals.
- To determine the resistant genes in *A. baumannii* isolates from Makkah Hospitals
- To isolate, purify and propagate the bacteriophage with lytic activity against of *A. baumannii.*
- To characterize the bacteriophage with lytic activity against *A. baumannii.*

1.4 Approach

The aim of this study was to identify the prevalent biotypes of *A. baumannii* clinical isolates and test their antimicrobial susceptibility to currently used antibiotics using Vitek 2 system from BioMérieux. Selected MDR isolates were further characterized for specific sequences type and resistant genes acquisition.

Searching for lytic bacteriophage was started in a few places at a few time intervals in hospital sewage systems to get bacteriophage candidates. Further characterization of isolated bacteriophages was planned by studying the genome of the isolated

bacteriophages by nucleic acid sequencing using Miseq NGS (next generation sequencing) system.

Current available bioinformatics tool such as Illumina Base space was used for the sequencing of the isolated bacteriophage. Mainly raw data from the illumina Miseq will undergo quality check using Galaxy or Basespace via FastQ. Next the good quality data was used for de novo assembly using application called DNAstar/Galaxy. The end product of de novo assembly in the form of contigs, which is a collection of overlapping DNA segments that collectively form a DNA consensus area and were used for further analysis via Center for genomic epidemiology. The gaps in the contigs were filled using linkers and then analyzed using PHASTER to find out known bacteriophages.

At the same time, the searching of bacteriophage will be done in sewage system of Makkah hospitals. Once the lethal bacteriophage has been isolated, electron microscopy was planned to be performed for identification of the bacteriophage and have a better idea about the morphology of the isolated bacteriophage.

All the research activities were carried out in the well-equipped molecular microbiology lab at Umm Al Qura University and the sample attained at tertiary care hospitals in Makkah. This study could present an already needed alternative treatment that has the potential of going into clinical trials. On the other hand, it can help us identify the most prevalent strains of *A. baumannii* that are present in the challenging hot environment. In a broader sense it could mean a chance of life for the critically ill patients as they would not have to bear with added burden of infection apart from their illness. Figure 1.2 shows the summary of approaches to achieve the objectives of the study.

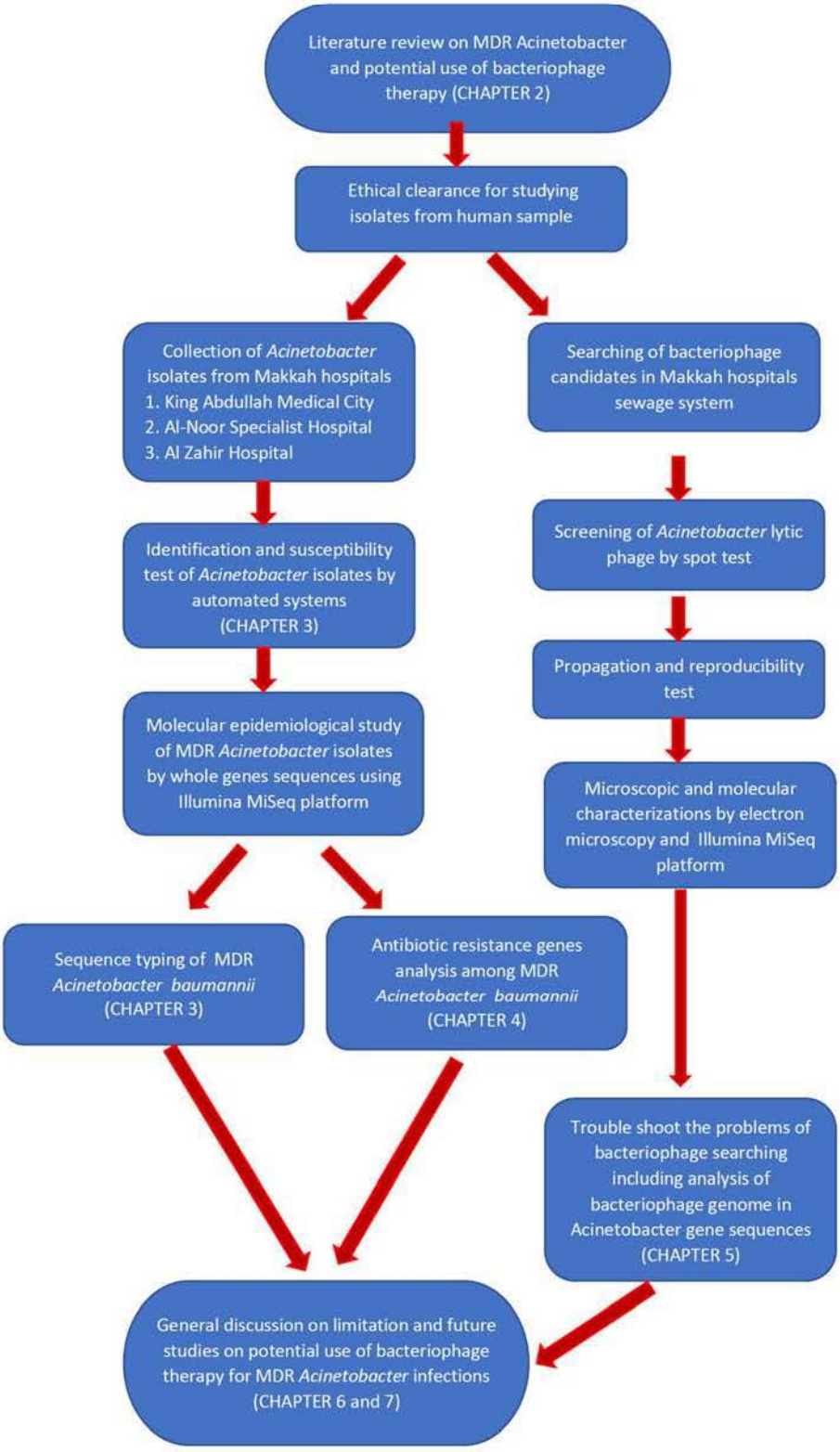

Figure 1.2 Approaches and summary of methodology to achieve objectives for this PhD project.

CHAPTER 2

LITERATURE REVIEW

2.1 *Acinetobacter baumannii*

2.1.1 History

In 1911 Beijerinck discovered the genus *Acinetobacter* and identified a pathogen which he called as *Micrococcus calcoaceticus* from the soil (Doughari *et al.*, 2011). It has been known by different names until 1950s when it was called as *Acinetobacter* (Munoz-Price and Weinstein, 2008). *Acinetobacter* were previously separated into different genera such as *Achromobacter anitratus, Alcaligenes haemolysans, Achromobacter mucosus, Diplococcus mucosus, Cytophaga, Bacterium anitratum, Herelleavaginicola, Mimapolymorpha, Lingelsheimia, M. calcoaceticus, Moraxella lwoffii* and *Neisseria winogradskyi* (Jung and Park, 2015). Over the past years the genus *Acinetobacter* has been repeatedly modified taxonomically, and the species *A. baumannii* was only officially named in 1986 (Antunes *et al.*, 2014). This genus is a component of *Moraxellaceae* family containing 21 species, with the most clinically significant one being *A. baumannii* (Abbott *et al.*, 2013). Taxonomically *Acinetobacter* spp. consists of *A. calcoaceticus–A. baumannii* complex (Abc) which comprises of: *A. calcoaceticus* (genomic species 1), *A. baumannii* (genomic species 2), *A. pittii* (genomic species 3) and *A. nosocomialis* (genomic species 13TU), which are very nearly related and identical (Abbott *et al.*, 2013).

In the 1970s *Acinetobacter* infections were mainly caused post-surgical urinary tract infections but since 1980s *A. baumannii* has spread in intensive care units (Joly-Guillou, 2005). Infection caused by *A. baumannii* was first recognized as a major issue in

the 1970s *(Bergogne-Berezin and Towner, 1996).* After the 1970s hospitalized patients have become susceptible to *A. baumannii* infections as a result of underlying conditions and advances in medical treatment such as antibiotics with a high selectivity, indwelling lines, and other invasive devices (Antunes *et al.*, 2014).

2.1.2 Bacteriology of *Acinetobacter baumannii*

A. baumannii is a Gram-negative, aerobic, nonmotile, catalase-positive, and oxidase-negative coccobacillus found mostly in hospitals, where it commonly colonizes IV and catheter lines of ICU patients (Howard *et al.*, 2012). It grows aerobically at an optimum temperature of 33–37°C on usual laboratory media. Moreover, it is non-motile, non-spore forming organism (Rahbar *et al.*, 2010).

A. baumannii can stay alive on dry surfaces due to its ability to grow at a range of temperature and pH with scarce nutritional requirement, for a period of five months. It can form biofilms that are resistant to various disinfectants on environment surfaces and medical devices such as catheters which aid in its ability to spread as a nosocomial pathogen (Nowak and Paluchowska, 2016). Thirty-one *Acinetobacter* species have been found using DNA hybridization, and they are loosely divided into 12 groups based on genetic similarity. Four of the 31 discovered species (*Acinetobacter calcoaceticus*, *Acinetobacter baumannii*, *Acinetobacter* genomic species 3 and 13TU) exhibit a similar phenotype that is difficult to differentiate in culture and are thus put together as the *A calcoaceticus-A. baumannii* complex (Acb) (Howard *et al.*, 2012), The majority of *Acinetobacter* species are found in the typical human skin flora (Sebeny *et al.*, 2008). *Acinetobacter* species are found in healthy populations at a frequency of up to 43%. However, *Acinetobacter* prevalence increases to up to 75% among the hospitalized population.

A. baumannii has evolved over the last several decades from a commensal organism seen in health-care settings to the most dangerous pathogen responsible for hospital outbreaks. Moreover, it is considered to be one of the most significant nosocomial pathogens. It was discovered to be the sixth most prevalent pathogen in an international survey of infections in critical care units covering five continents (Roca Subirà *et al.*, 2012). These are probably due to:

(i) *A. baumannii* possesses an exceptional capacity for accumulating a diverse array of resistance mechanisms via a variety of mechanisms, including mutations, acquisition of genetic elements such as plasmids, integrons, transposons, or resistant islands, thereby conferring multi- or pan-drug resistance on this microorganism.

(ii) Because of its capacity to survive and tolerate the environment for extended periods of time, along with its inherent resistance to desiccation and disinfectants, *A. baumannii* is nearly hard to treat in a clinical setting.

(iii) Additionally, its capacity to form biofilms significantly increases its persistence and resistance (Roca Subirà *et al.*, 2012).

2.1.3 Virulence factors

Until now, very few virulence factors (VF) for *A. baumannii* have been identified which may increase with further studies with genome sequencing and animal model studies (Nowak and Paluchowska, 2016).

Lipopolysaccharide (LPS), capsular polysaccharides (CPS), *A. baumannii* outer membrane protein A (AbOmpA), outer membrane vesicles (OMV), phospholipase D (PLD), and biofilm are all virulence factors that contribute to *A. baumannii's* pathogenicity (Roca Subirà *et al.*, 2012; McConnell *et al.*, 2013). Capsular polysaccharides, such as the

K1 capsule isolated from the clinical strain *A. baumannii* AB307-0294, have also been implicated in protecting the bacteria both in vivo and in vitro by providing protection against the complement system, a possibility that should be researched further (Russo *et al.*, 2010).

AbOmpA is one of the well-characterized virulence factors causing damage to the human airway cells mediated by proapoptotic molecule cytochrome c and apoptosis-inducing factor. Moreover, AbOmpA may have a role in adherence, invasion of epithelial cells dissemination as well as surface motility, resistance to complement and biofilm formation (Choi *et al.*, 2005; Choi *et al.*, 2008; Roca Subirà *et al.*, 2012).

2.1.4 Clinical relevance and presentations

As described in 2005, *A. baumannii* are opportunistic bacterial pathogens that account for between 2% and 10% of all Gram-negative hospital infections (Joly-Guillou, 2005). *A. baumannii* is responsible for many acute hospital infections such as respiratory infections especially ventilator-associated pneumonia (VAP), urinary tract infections, bloodstream infections, infections of skin and soft tissue, burn and surgical wound, endo-carditis, meningitis, and osteomyelitis (Antunes *et al.*, 2014). Surgery, major trauma, neonates or old age, hospital stay, use of antimicrobial and mechanical ventilation, intravascular and urinary catheters are the major risk factors that lead to colonization and infection of patients with *A. baumannii* with the risk of mortality between 8% to 40% (Dijkshoorn *et al.*, 2007; Lemos *et al.*, 2014). In the tropical regions *A. baumannii* has been reported to be the causative agent for community acquired infection such as pneumonia and bacteremia especially in susceptible individuals who have other comorbidities like diabetes mellitus, renal failure or chronic obstructive pulmonary

disease (Falagas *et al.*, 2007; Karageorgopoulos and Falagas, 2008). When community acquired *A. baumannii* pneumonia does develop, it follows a rapid course leading to fever, respiratory symptoms and failure or organs with a mortality rate reaching 64% (Dexter *et al.*, 2015). *A. baumannii* infection frequently results in hospital-wide epidemics and rather significant fatality rates. *A. baumannii* is the most commonly isolated organism among other *Acinetobacter* species in clinical samples (Turton *et al.*, 2010). *Acinetobacter* infection is a significant source of hospital-acquired illness globally, facilitated by its ability to persist on surfaces and equipment for extended periods of time. The mortality associated with *A. baumannii* infection in the intensive care unit (ICU) setting can approach 40% (Alsan and Klompas, 2010). *A. baumannii* is responsible for up to 10% of all Gram-negative ICU infections in North America and Western Europe. Along with high morbidity rates and increased costs to the hospital care system, *A. baumannii* is directly responsible for considerably longer patient hospital stays and much higher hospital care expenses.

A. baumannii has emerged as a major cause of hospital-acquired illnesses worldwide. The present clinical spectrum of *A. baumannii* infections is mostly comprised of nosocomial infections, such as pneumonia acquired in a critical care unit, blood stream infections, and urinary tract infections, meningitis and in rare cases, endocarditis (Nunez *et al.*, 1998; Wisplinghoff *et al.*, 2004; Gaynes and Edwards, 2005; Olut and Erkek, 2005; Metan *et al.*, 2007). Additionally, *A. baumannii* has been reported to cause community-acquired pneumonia in certain tropical climates (Anstey *et al.*, 2002; Leung *et al.*, 2006). *A. baumannii* was also implicated in cases infected traumatic wounds acquired in battlefield conditions (Murray *et al.*, 2006; Johnson *et al.*, 2007; Petersen *et al.*, 2007). *A. baumannii* infections have been reported from war conflict zones such as Afghanistan,

Iraq and Syria or areas that have suffered from natural disaster such as earthquakes and tsunami advocated in some studies to the pressure on the hospitals involved (Nowak and Paluchowska, 2016).

A. baumannii infections morbidity and mortality are well documented. For instance, *A. baumannii* ICU-pneumonia is usually encountered in 5–10% of patients receiving mechanical ventilation (Gaynes and Edwards, 2005). More than 35% of ICU patients die because of *A. baumannii* (Johnson et al., 2007) bloodstream infections (Wisplinghoff *et al.*, 2004). Community-acquired pneumonia affects alcoholics in tropical regions and result in high mortality rates (Leung *et al.*, 2006). *A. baumannii* causes skin/soft tissue infections in ICU patients and has been isolated from large number of soldiers with infected wounds (Gaynes and Edwards, 2005).

2.1.5 Antimicrobial resistance

The alarming drop in *A. baumannii* susceptibility to several antimicrobial agents, including carbapenems, underscores the critical need for novel therapeutic options. Additionally, *A. baumannii* presents a distinct problem in hospitals and therapeutic settings due to its great resilience to harsh circumstances.

A. baumannii was cultivated from 72% of bronchoalveolar lavage fluid (related with ventilation) collected from 291 patients in American internal care units in recent retrospective research. Two isolates were found to be resistant to all antibiotics tested, 81% were found to be resistant to the β-lactam imipenem-cilastatin, and one isolate was found to be resistant to all antibiotics except colistin (polymyxin B) (Trottier *et al.*, 2007).

MDR *A. baumannii* creates a great challenge in clinical practices worldwide. It has emerged as one of the most problematic causative agents of hospital-related infections

worldwide (Peleg *et al.*, 2008). Naturally environmental organism, *Acinetobacter* can be part of the human skin flora. *A. baumannii* is the most important nosocomial *Acinetobacter species* (Peleg *et al.*, 2008) and the most resistant to antimicrobial agents (Wong *et al.*, 2017). There are wide range of clinical spectrum of the infections, from less severe urinary tract infections, wound infections and pneumonia to severe form blood stream infection, meningitis and carditis (Bergogne-Berezin and Towner, 1996; Peleg *et al.*, 2008; Wong *et al.*, 2017; Moubareck and Halat, 2020).

The primary difficulty in treating *Acinetobacter* infections is due to the acquiring antimicrobial resistance. The rapid global emergence of *A. baumannii* strains resistant to virtually all available antimicrobial agents is quite alarming (Peleg *et al.*, 2008; Wong *et al.*, 2017; Moubareck and Halat, 2020) *Acinetobacter* expresses almost all range of resistant mechanisms include very small number and size of porins, active efflux systems, absence of PBP2 and possesses all classes of β-lactamases. The resistance island in its genome comprised of 45 resistance genes (Wong *et al.*, 2017).

The resistance to the most active antimicrobial agents against Gram-negative organisms, the carbapenems, can be resulted in reduced outer membrane porin that lead to low permeability to carbapenems, production of naturally occurring oxacillinases (OXA-23, OXA-24 or -40, OXA-51, OXA-58, and OXA-143), absence of PBP2 and the most robust one, possesses class B β-lactamases (metallo-β-lactamases) (Wong *et al.*, 2017). As *Acinetobacter* develop more and more resistance to antimicrobial agents, with some strains are virtually resistant to all available agents, the treatment options for *Acinetobacter* are very limited. One of the remaining potential agents is bacteriophage.

2.1.6 Mechanisms of Antimicrobial Resistance in *A. baumannii*

Antimicrobial resistance in *A. baumannii* is mediated by all of the primary resistance mechanisms known to occur in bacteria, including target site alteration, enzyme inactivation, active efflux, and reduced drug inflow (Peleg *et al.*, 2008) and carbapenem resistance is the good example (Poirel and Nordmann, 2006). *A. baumannii* multidrug resistance has been recently linked to the presence of large amount of monovalent cation in skin (Hood *et al.*, 2010). *A. baumannii* able to produce metallo-β-lactamases, which have been reported to confer resistance to most β-lactam antibiotics (Lee *et al.*, 2003; Lee *et al.*, 2005). Many antibiotic resistant markers have been described for *A. baumannii,* However, most of these markers are also found within other genera as well, specifically Gram-negative bacteria, and few are unique for the genus *Acinetobacter* (Mihu and Martinez, 2011).

The mechanism of carbapenem resistance can be attributed to the chromosomally driven upregulation of the blaOXA-51-like β-lactamase gene and acquiring more OXA-carbapenemases, acquiring metallo-β-carbapenemase of class B and other enzymatic mechanisms include the TEM, CTX-M, VEB, PER, and GES families of class A ESBLs. The resistance is also can be due to penicillin-binding proteins are altered, porin proteins are altered, and efflux pumps are up-regulated (Perez *et al.*, 2007; Gordon and Wareham, 2010).

In *A. baumannii*, the efflux pump is also responsible for resistance to aminoglycosides, quinolones, tetracyclines, chloramphenicol, erythromycin, and trimethoprim (Nowak *et al.*, 2015).

2.1.7 Genotyping of *A. baumannii*

Especially in nosocomial pathogens, the sources of the infecting organisms and their clonality, are a necessary condition for the development of effective infection control methods. Genotyping enables the analysis of clonal spread and can be used to pinpoint the origin of an illness. Many DNA-based methods have been used for the genotyping of *Acinetobacter* strains. Repetitive extragenic palindromic sequence-based PCR and arbitrary primer sequence-based PCR techniques were utilized and compared to pulsed-field gel electrophoresis (PFGE) as the standard genotyping technique (Bou *et al.*, 2000; Huys *et al.*, 2005). Many other DNA-based techniques have been used for *A. baumannii* genotyping and/or identification (Vaneechoutte *et al.*, 1995a; Vaneechoutte *et al.*, 1995b; Ibrahim *et al.*, 1997; Koeleman *et al.*, 1998; Webster *et al.*, 1999; Houang *et al.*, 2001; Spence *et al.*, 2002; van Dessel *et al.*, 2002; Misbah *et al.*, 2005).

2.1.8 Treatment options

Resistance in *A. baumannii* has limited the treatment options, and the rapid dissemination of multidrug resistance leads to the use of antibiotics without appropriate evidence. Some investigators suggested carbapenems as treatment of choice (Maragakis and Perl, 2008). Other investigators found sulbactam is beneficial in the treatment of infections of the bloodstream, respiratory tract, and urinary tract (Levin *et al.*, 2003; Smolyakov *et al.*, 2003), although the contribution of ampicillin is negligible (Corbella *et al.*, 1998). Comparable safety and efficacy were reported when high dose of ampicillin/sulbactam versus colistin were evaluated for the treatment of ventilator associated pneumonia (Betrosian *et al.*, 2008). Additionally, carbapenem-sulbactam

combinations have been proven to be effective against carbapenem-resistant isolates (Ko *et al.*, 2004; Lee *et al.*, 2007).

The majority of MDR *A. baumannii* strains remain susceptible to polymyxins, which result in wide use of this toxic antibiotics (Falagas and Kasiakou, 2005). Colistin (polymyxin E) was effectively used for the treatment of bloodstream, wound and urinary tract infections (Gounden *et al.*, 2009). Tigecycline, which is derivative of minocycline, is effective in the management of complicated skin and soft-tissue infections (Ellis-Grosse *et al.*, 2005) and intra-abdominal infections (Oliva *et al.*, 2005) and show good in vitro activity against multi-drug resistant *A. baumannii*.

MDR *A. baumannii* has resulted in attempts for the use of combination of two or even three antibiotics, without in vitro significant synergy (Ko *et al.*, 2004). Colistin has been coupled with rifampicin, minocycline, ceftazidime, or imipenem without demonstrating any clinical advantage, while sulbactam has been mixed with meropenem without demonstrating any therapeutic benefit (Petrosillo *et al.*, 2008). Tigecycline in combinations with colistin, levofloxacin, amikacin and imipenem showed some synergy, but an antagonism was reported when tigecycline was combined with piperacillin/tazobactam (Principe *et al.*, 2009).

2.2 *Acinetobacter baumannii* in Saudi Arabia

2.2.1 Epidemiology of antimicrobial resistance is Saudi Arabia

Saudi Arabia is divided into 14 administrative regions (See Figure 1.1). The Kingdom of Saudi Arabia, as it is officially known, is located in Western Asia. It is the Middle East's largest sovereign state and economy, covering a territory of approximately

2,150,000 kilometers square. Saudi Arabia was historically separated into four regions: Hejaz, Najd, and sections of Eastern Arabia (Al-Ahsa) and Southern Arabia (Asir), with a population of 33 million in 2017, primarily concentrated in major cities. Additionally, Saudi Arabia hosts major religious gatherings of up to 3 million Muslims from around the world each year, dubbed the "Hajj pilgrimage" during which infectious disease transmission is possible. Due to the foregoing, Saudi Arabia might serve as a hub for the interchange of MDR strains from around the world (Memish *et al.*, 2012b; Yezli *et al.*, 2014).

A. baumannii is a common pathogen isolated in Saudi hospitals and responsible for 11%-28% of Gram-negative isolates as reported from two major tertiary-care hospitals in Saudi Arabia (Asghar and Faidah, 2009; Saeed *et al.*, 2010; Yezli *et al.*, 2014). In addition, the geographic location, ethnic diversity, migration from Indian subcontinent, in Saudi Arabia who accommodates greater than 1.5 million international pilgrims from across the globe is an important risk factor for distribution of antimicrobial-resistant bacteria (Memish, 2010). The most important factor responsible for development of antibiotic resistance is inappropriate use (Luyt *et al.*, 2014). Table 2.1 shows the prevalence of *A. baumannii* in Saudi Arabia as reported in previous publications.

Table 2.1 Prevalence of *A. baumannii* reported from Saudi Arabia

Year of study	Place of study (No of isolates)	Location of study	Prevalence	Proportion of resistance	Reference
1998-2004	Dhahran (476)	Whole hospital	Approximately 5% of total isolates	35.8% of *Acinetobacter* were MDR	(Al-Tawfiq and Mohandhas, 2007)
2001	Jeddah (499)	Whole hospital	34% of GNB	NA	(Eltahawy and Khalaf, 2001)
2004-2005	Makkah (1626)	Whole hospital	7.4% of total isolates	14% resistant to imipenem	(Asghar, 2006)
2005-2006	Makkah (1137)	Whole hospital	10.8% of GNB	45.9 % of patients were resistant to imipenem, whereas 28% were resistant to meropenem.	(Asghar and Faidah, 2009)
2009	Riyadh (1210)	Intensive care unit	19.4% of total isolates	97.0%of *Acinetobacter* were MDR	(Saeed *et al.*, 2010)
2009	Nationwide (8908)	Whole hospital	25.3% of non-fermenting GNB	5.4% resistant to imipenem	(Memish *et al.*, 2012a)
2010	Taif (170)	Whole hospital	12% of respiratory isolates	NA	(Sabra and Abdel-Fattah, 2012)
2010-2012	Hofuf (758)	Intensive care unit	31.9% of total isolates	91.7% of *Acinetobacter* were MDR	(Mwanri and Alsaleh, 2014)
2013	Riyadh (1307)	Whole hospital	NA*	Resistance to tigecycline is 9.7%, and to colistin is 1.8 %.	(Baadani *et al.*, 2013)
2010-2013	Jeddah (1176)	King Abdul Aziz university hospital	4.2% (2010) to 12.3% (2013)	NA	(Al Mobarak *et al.*, 2014)
2011	Makkah and Jeddah (72)	Whole hospital	NA	62.5% resistant to imipenem	(Khan *et al.*, 2012)
2012	Nationwide (242)	Whole hospital	32.7% of GNB	100% resistant to carbapenem	(Memish *et al.*, 2015)
2012-2014	Makkah (107)	Intensive care unit	NA*	94% of *Acinetobacter* were MDR	(Alyamani *et al.*, 2015)

2013	Riyadh (457)	Intensive care unit	26.5% of total isolates	NA	(El-Saed *et al.*, 2013)
2013	Najran (125)	Whole hospital	54.5% of GNB	7.4% of *Acinetobacter* were resistant to imipenem. 0% resistant to colistin	(Asaad *et al.*, 2013)
2014	Dammam (565)	Intensive care unit (Rectal swab screening)	8.3% of the samples	74.5% of *Acinetobacter* sp. were carbapenem resistance	(Aljindan *et al.*, 2015)
2014-2015	Asir (94)	Whole hospital	NA*	69% of *A. baumannii* were MDR. 36.2% were PDR (susceptible only to colistin)	(Almaghrabi *et al.*, 2018)
2015	Makkah (374)	Whole hospital (during Hajj)	7% of the total isolates	90% were resistant to imipenem and 64% resistant to meropenem	(Haseeb *et al.*, 2016)
2015	Al Ahsa (4532)	Hospital	20% of total isolates	20% resistant to imipenem and 44% resistant to meropenem	(Ahmed *et al.*, 2015)
2016	Asir (105)	Intensive care unit	NA*	98.1% were MDR but all were susceptible to colistin	(Al Bshabshe *et al.*, 2016)
2016	Riyadh (56)	Oncology unit	18% of GNB	81.8% were resistant to meropenem and 73.7% resistant to imipenem	(Al-Otaibi *et al.*, 2016)
2016	Madinah (6840)	Whole hospital	5.5% of total isolates	89.2% resistant to imipenem	(Ghanem *et al.*, 2018)
2016-2018	Bisha Province (290)	Intensive care unit	27.2% of GNB	97.5% were MDR and 4.0% were resistant to colistin	(Ibrahim, 2018)

2.2.2 Antibiotic resistance among *Acinetobacter* in Saudi Arabia

Antimicrobial resistance has increased in Saudi Arabia for a variety of causes. For example, readily available broad-spectrum antibiotics, including 3rd and 4th generation cephalosporins, quinolones, and carbapenems. The lack of antimicrobial stewardship programs and the absence of strong infection control programs as well as proper human resources led to uncontrollable transmission of the MDR strains. In addition to that, hospitals with old architectural design of 2 and 4 bedded rooms make difficult for patient with MDR organism difficult to be isolated (Aly and Balkhy, 2012).

A research done in a hospital in Saudi Arabia's Aseer area discovered that 98.1 % of *Acinetobacter* species were multidrug resistant, while 100 percent were susceptible to colistin, and 74.5 % were susceptible to trimethoprim/sulfamethoxazole (Al Bshabshe *et al.*, 2016). Moreover, *A. baumannii* used to be susceptible to many antibiotics until 1970s (Fournier *et al.*, 2006)

Current reports from Saudi Arabia show a significant rise in the number of resistant and MDR agents like *A. baumannii* (Lakshmana Gowda *et al.*, 2014). Over 75% of isolates were resistant to several classes of antibiotics, including colistin, meropenem, imipenem, and trimethoprim/sulfamethoxazole (95.6, 50, 48.1, and 34.3 percent, respectively). Al Bshabshee al. 2016 reported 98.1% of *Acinetobacter* species were found to be MDR in Aseer region, however, they were susceptible to colistin (100%) and trimethoprim/sulfamethoxazole (74.5%) (Elabd *et al.*, 2015). Over a two-year period from 2012 to 2014, the implementation of antibiotic stewardship programs shown that these programs might reduce the dispensation and prescription of restricted antibiotics by 67% and 75%, respectively (Alawi and Darwesh, 2016).

Globally, awareness must be raised among physicians and patients to improve antibiotic prescription and use in order to avert an alarming future (Alawi and Darwesh, 2016). In the setting of Saudi Arabia more focus needs to be put on the implementation of the guidelines, as good level of awareness exists among physicians as well as national guidelines (Baadani *et al.*, 2015).

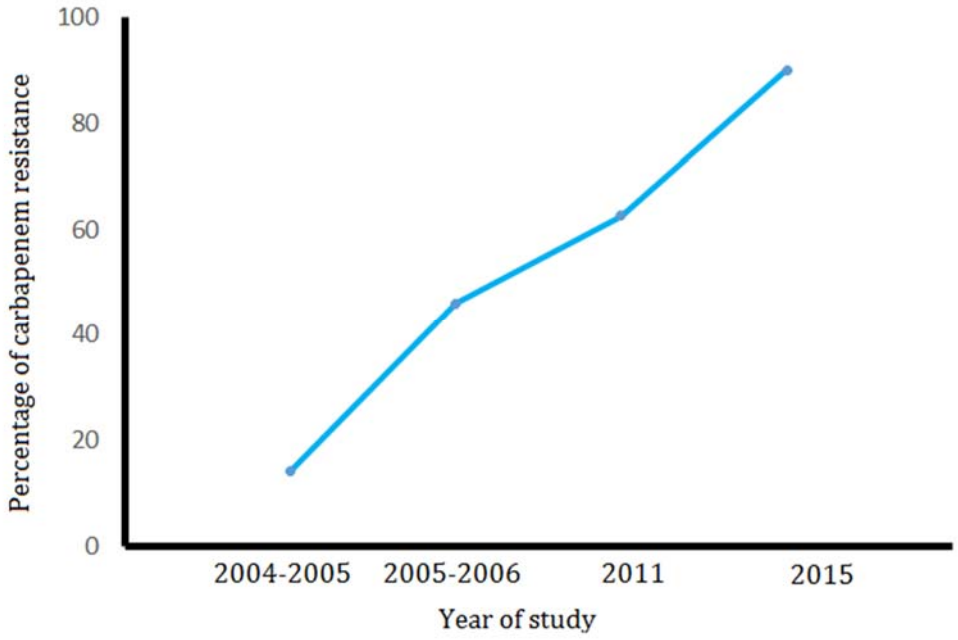

Figure 2.1 An increasing trend of the proportion of *Acinetobacter* resistant to carbapenem in Makkah from 2004 to 2015

2.2.2 (a) Penicillin

Between 1994 and 2010 75% of *A. baumannii* throughout the Kingdom were resistant to ampicillin. While sulbactam has intrinsic activity, 63% of isolates from a Riyadh critical care unit in 2009 were ampicillin/sulbactam resistant, compared to 93% for piperacillin/tazobactam. Between 1998 and 2004, ticarcillin and its combination with clavulanic acid were effective against a large number of *Acinetobacter* collected at Saudi Aramco Medical services organization (Al-Tawfiq and Mohandhas, 2007), Aztreonam is

intrinsically ineffective against *Acinetobacter*, with over 83% of isolates proved resistant in previous publications (Saeed *et al.*, 2010; Memish *et al.*, 2012a). According to more recent research, in general, a significant level of resistance to β-lactam antibiotics was identified (Haseeb *et al.*, 2016). In 2018, a research conducted in Madinah, Saudi Arabia, found resistance to amoxycillin/clavulanate and piperacillin were at 99.21 % and 97.63 %, respectively (Ghanem *et al.*, 2018).

2.2.2 (b) Cephalosporins

Acinetobacter is mainly resistance to first and second generation cephalosporins, but to some extent, there are variable activities against third generation cephalosporins. Between 1994 and 2009, 86 percent of *Acinetobacter* isolates in Riyadh were ceftriaxone resistant. In addition, research from Riyadh, Al-Khobar, and Madinah indicated that cefotaxime resistance was 50% and ceftazidime resistance was 88 % (Qadri *et al.*, 1995; Al-Tawfiq and Mohandhas, 2007; Saeed *et al.*, 2010; Alsultan *et al.*, 2013). A few other studies also indicated same high resistance rates. Resistance rates varied from 58 percent in the Eastern and the Western region, to 72.5% in Aseer and to up to 95.8% in Hail (Memish *et al.*, 2012a). More recently, further increase in resistance rates has been reported to be up to 99.21%, 99.74%, 98.42% for cefotoxin, cephalothin and ceftazidime respectively (Ghanem *et al.*, 2018).

2.2.2 (c) Carbapenem

The resistance to carbapenems has been shown to be more common in the Middle East and in Saudi Arabia (Wang and Dowzicky, 2010; Alsultan *et al.*, 2013). The earlier report from Saudi Arabia indicated a very low rate of resistance against carbapenem. From 1998-2004 the rate of imipenem resistance was found to be at 3% only (Al-Tawfiq and

Mohandhas, 2007). In 2009, a countrywide assessment of 2228 *A. baumannii* isolates revealed that 5.4% of isolates were resistant to imipenem, with the Eastern area reporting the highest prevalence of 13.1 percent (Memish *et al.*, 2012a). After that, carbapenem resistant *A. baumannii* from Saudi Arabia appears to have risen over the years. The emergence of *A. baumanii* strains that resistant to all clinically available antibiotics except colistin were detected in 2012 led to a major concern in clinical practice (Al-Obeid *et al.*, 2015). Alsultan et al. (Alsultan et al., 2009; Alsultan et al., 2013) suggested that VIM, OXA-23, OXA-40, OXA-89, and OXA-66 carbapenemases, as well as new chromosomal OXA-51-like β-lactamases, were involved in the mechanism of carbapenem resistance.

In 2014 a study done in Dammam, Saudi Arabia reported 32.6% of the *A. baumannii* isolated to be carbapenem resistant with underlying mechanism related to *bla*$_{\text{OXA-23}}$ (Abdalhamid *et al.*, 2014). Whereas higher rates of carbapenem resistance or intermediate resistance in 69% of *A. baumannii* isolates have also been reported with concomitant *bla*$_{\text{VIM}}$ gene detection in 94%, while *bla*$_{\text{OXA-23-like}}$ genes in 58% in Eastern District of Saudi Arabia (Al-Sultan *et al.*, 2015). High rates of resistance have also been reported in the southern region of Saudi Arabia citing a new threat in the hospitals reporting 69% multi drug resistant isolates (Almaghrabi *et al.*, 2018). A study in Riyadh has found 76.3 % prevalence of the PER-1 resistance gene in *A. baumannii* clinical isolates (Aly *et al.*, 2016). Most *A. baumannii* strains were found to be resistant to imipenem 90.5%, meropenem 90.5%, and doripenem 77.4% (Somily *et al.*, 2012). The acquisition of resistance to carbapenems in *A. baumannii* has been attributed to a number of mechanisms including the expression of OXA-type A and metallo-β-lactamase (Evans *et al.*, 2013). More specifically reports on isolates from the Arabian gulf exhibit that

carbapenem resistance phenotype in *A. baumannii* is mostly because of the expression of OXA enzymes, and in particular OXA-23 (Zowawi *et al.*, 2013). It is particularly noteworthy that the imipenem resistance rate has increased to 89.18% in 2018 (Ghanem *et al.*, 2018).

2.2.2 (d) Aminoglycosides

Resistance to aminoglycosides has grown over time in *A. baumannii*. From the 1980s to the early 2000s, resistance rates of around 40% were documented (Moaz *et al.*, 1989; Kader *et al.*, 2004; Al-Tawfiq and Mohandhas, 2007; Al-Tawfiq and Abed, 2009) whereas recent reports suggest that over 75% of the isolates are now resistant to gentamicin and amikacin, with 47% resistant to netilmicin. Resistance to amikacin and gentamicin was highest in Makkah around 90% and lowest in the Eastern region around 60% (Al Johani *et al.*, 2010; Saeed *et al.*, 2010; Memish *et al.*, 2012a). In 2012 the reported rate of resistance for amikacin was 76.9% and for gentamicin was 77.8% (Memish *et al.*, 2012a). More recent studies from 2016-2018 have report resistance to amikacin to be 67% and 83.7% respectively (Al Bshabshe *et al.*, 2016; Haseeb *et al.*, 2016).

2.2.2 (e) Polymyxins

Colistin is a cationic polypeptide and a member of the polymyxin family is the last resort in the battle against multi drug resistant *A. baumannii.* Previously colistin resistance was not reported and in 2013 a study from Najran, Saudi Arabia reported 100% (all 68 isolates) of the isolates to be susceptible to colistin (Asaad *et al.*, 2013). Up until 2015 all isolates of *A. baumannii* were found to be sensitive to colistin (Marie *et al.*, 2015). Similar results were reported by a study in Aseer region (Al Bshabshe *et al.*, 2016). In spite of the above colistin with or without rifampin appears to be the best available option (Abbott *et al.*, 2013). In one investigation, 74% of isolates were found to be multidrug resistant, with