UNIVERSITI PUTRA MALAYSIA

MOLECULAR EPIDEMIOLOGY OF MULTIPLE DRUG RESISTANT Acinetobacter baumannii FROM A MAJOR TEACHING HOSPITAL IN MALAYSIA

MOHAMMAD REZA BABAEI MOGHADDAM

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

December 2016

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DEDICATION

In the name of God the Copassionate the merciful

To my beloved son *Amir Mahdi* and my beloved wife *Neda Alavi* and my family for invaluable support and extraordinary courage

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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Chairman : Associate Professor Vasantha Kumari Neela, PhD
Faculty : Medicine and Health Science

Acinetobacter baumannii is a Gram-negative opportunistic coccobacilli, the most important agent in nosocomial infections with high mortality rate. Multidrug resistance in strains isolated from nosocomial infections, makes it difficult and sometimes impossible to treat. The aim of this study is to investigate the molecular epidemiology of Multiple Drug Resistant *Acinetobacter baumannii* (MDRAB) in a tertiary care hospital in Malaysia (UKMMC).

During the study period a total of 18509 patients were admitted at different wards in the hospital. Among them, 122 patients were infected with MDRAB comprising 56.5% males and 43.5% were females. The highest number of infection was observed in medical ward (n=31, 25.4%), followed by ICU (n=30, 23.6%), surgery (n=28, 22.9%), orthopaedic (n=11, 9%), urology (n=6, 4.9%), neurosurgery (n=4, 3.2%), burn (n=3, 2.5%)

All isolates were phenotypically and genotypically confirmed as *A. baumannii*. All 122 isolates were screened for 25 antibiotics. Among the 25 antibiotics tested, except for polymixin B, all isolates showed resistance to more than 3 antibiotics. Imipenem and meropenam showed 100% resistance. A total of 14 antibiotic resistance genes were screened. The resistances for genes ranged from 1.6 to 99.9% for different antibiotics such as quinolones (*ParC* 97.5%; *gyr* 97.5%), cephalosporin (*TEM* 95.9%; *CTX-M* 1.6%; *PER* 93.4%) and carbapenems (OXA_{58} 84.6%; OXA_{51} 99.2%, OXA_{23} 97.5%, *IMP* 2.45%, *NDM-1* 1.6%).

Antiseptics are commonly used for the management of MDR (multiple drug resistance) pathogens in hospitals. They play crucial roles in the infection control practices. Antiseptics are often used for skin antisepsis, gauze dressing, and

preparation of anatomical sites for surgical procedure, hand sterilization before in contact with an infected person, before an invasive procedure and as surgical scrub. All 122 MDRAB isolates screened for the presence of antiseptic resistant genes *QacA/B* and *QacE* (Quaternary Ammonium Compounds) and susceptibility towards chlorhexidine (CLX), benzalkonium (BZK) and benzethonium (BZT).

Eighty-nine (73%) isolates harboured *QacE* gene, while none were positive for *QacA/B*. The MIC ranged from 0.2 to 0.6 for CLX, 0.02 to 0.2 for BZK and 0.04 to 0.2 μg/mL for BZT. The highest number of *QacE* positive isolates were obtained from surgery (n = 24; 27%; $p < 0.05$), followed by medical ward (n = 23; 25.8%) and ICU (n = 21; 23.6%). Majority of the isolates from wound swabs (n = 33; 37%), T/aspirate (n = 16; 18%) and tissue (n = 10; 11.2%) harboured the *QacE* genes.

Followed by antibiotic and antiseptic susceptibility testing, all isolates were subjected to genomic fingerprinting through pulsed field gel electrophoresis (PFGE) approach. Analysis demonstrated seven clusters (A-B-C-D-E-F and G) with a high level of diversity among different isolates and wards. The degree of resistance for 25 antibiotics was compared among all seven clusters and the results indicated a significant difference among these groups ($p=0.019$). The highest resistance was observed in cluster C (23.667 – carbapenems) while the lowest belonged to both clusters B (22.000 – cephalosporins) and F (22.000 – quinolones).

Antimicrobial resistance pattern for different isolates with same resistance were in different clusters and the highest resistance was observed in three main antibiotic classes. *QacE, QXA_{58}* and *PER* had the highest variation. The range of frequencies for *QacE* was between 100% (cluster B) and 42.9 % (cluster F), and this range for *OXA_{58}* was between 94.4% (cluster A) and 28.6% (cluster F), and for *PER* was between 100% (cluster B) and 66.7% (cluster C). In other words, these three genes had higher variation among all samples and consequently more contribution to form these clusters.

Biofilms and quorum sensing (QS) are two phenomena that are very important and crucial in helping the MDRAB to cling to the skin, mucosal, and surfaces of inanimate objects such as medical devices (ventilator and tracheal aspirate) in hospital environments. Biofilms are known to increase drug resistance in bacteria. Quorum sensing (QS) characteristic in bacterial allows it to monitor their population density through the production and sensing of small signal molecules known as auto inducers. In MDRAB auto inducers are acyl-homoserine lactones (AHLs), (Chenia, 2013). The strong association between antibiotic resistance genes and QS through signalling pathway has been well established. In the present study, biofilm formation in selected highly resistant MDRAB isolates were analysed and were screened for the QS *abaI* gene. The biofilm formation increased 2-4 folds in resistant MDRAB and were significantly higher compared to the control *Escherichia coli* strain.

In conclusion, MDRAB is more common in ICU, resistant to most antibiotics available for treatment including carbapenems with high prevalence several antibiotic resistant genes. Although high prevalence of *QacE* gene responsible for antiseptic resistance was observed, the MIC was much less than the concentration used in the hospitals. Highest genome diversity among the MDRAB isolates cleary shows that the isolates are highly diverse, however resistance are homogenous. Presence of *abaI* gene in MDRAB genes that produce high concentration of biofilms indicates these strains needs more attention due to its adherence to the hospital environment and surfaces on inanimate objects such as catheters, shunts and ventilators. Regular monitoring for antibiotic and antiseptic resistance in wards are important for the effective management of MDRAB strains in the hospital.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

EPIDEMIOLOGI MOLEKULAR *Acinetobacter baumanii* RESISTANT TERHADAP PELBAGAI ANTIBIOTIK DI HOSPITAL PENGAJAR UTAMA DI MALAYSIA

Oleh

MOHAMMAD REZA BABAEI MOGHADDAM

Disember 2016

Pengerusi : Profesor Madya Vasantha Kumari Neela, PhD
Fakulti : Perubatan dan Sains Kesihatan

Acinetobacter baumannii ialah coccobacilli oportunis Gram-negatif, ejen yang paling penting dalam jangkitan nosokomial yang menyebabkan kadar kematian yang tinggi. Perintang pelbagai dadah ini diasingkan daripada jangkitan nosokomial, menjadikan ia sukar dan kadangkala mustahil untuk dirawat. Tujuan kajian ini adalah untuk menyiasat epidemiologi *Acinetobacter baumannii* perintang pelbagai dadah (MDRAB) di hospital pengajian tinggi di Malaysia (PPUKM).

Dalam tempoh kajian sejumlah 18.509 pesakit dari wad yang berbeza di hospital dikaji. Antaranya, 122 pesakit dijangkiti MDRAB terdiri daripada 56.5% lelaki dan 43.5% adalah perempuan. Bilangan tertinggi jangkitan diperhatikan di wad perubatan (n = 31, 25.4%), diikuti oleh ICU (n = 30, 23.6%), pembedahan (n = 28, 22.9%), ortopedik (n = 11, 9%), urologi (n = 6, 4.9%), pembedahan saraf (n = 4, 3.2%), kebakaran (n = 3, 2.5%)

Semua pencilan telah disahkan secara fenotipik dan genotipik sebagai *A. baumannii*. Semua 122 pencilan telah disaring untuk 25 antibiotik. Antara 25 antibiotik diuji, semua pencilan menunjukkan rintangan kepada lebih daripada 3 antibiotik kecuali polymyxin B. Imipenem dan meropenam menunjukkan rintangan 100%. Sebanyak 14 gen rintangan antibiotik telah disaring. Rintangan untuk gen adalah seperti berikut; Quinolones (*ParC* 97.5%; *gyr* 97.5%), cephalosporin (*TEM* 95.9%; *CTX-M* 1.6%; *PER* 93.4%) dan carbapenems (*OXA58* 84.6%; *OXA51* 99.2%, *OXA23* 97.5%, *IMP* 2.45%, *NDM* 1.6 %).

Antiseptik biasa digunakan bagi pengurusan patogen MDR (pelbagai rintangan dadah) di hospital. Ia memainkan peranan penting dalam amalan kawalan jangkitan. Antiseptik sering digunakan untuk antisepsis kulit, kain kasa, dan penyediaan laman anatomi bagi prosedur pembedahan, pensterilan tangan sebelum bersentuhan dengan orang yang dijangkiti, sebelum prosedur invasif dan sebagai penyental pembedahan.

Semua 122 pencilan MDRAB menunjukkan kehadiran gen tahan antiseptik *QacA / B* dan *QacE* (QuaterAmmonium Compound) dan sensitif terhadap chlorhexidine (CLX), benzalkonium (BZK) dan benzethonium (BZT).

Lapan puluh sembilan (73%) pencilan membawa gen *QacE*, manakala tiada positif untuk *QacA / B*. MIC adalah antara 0,2-0,6 untuk CLX, 0,02-0,2 untuk BZK dan 0,04-0,2 μg / mL untuk BZT. Pencilan positif tertinggi QacE telah diperolehi daripada wad pembedahan (n = 24; 27%; p <0.05), diikuti dengan wad perubatan (n = 23; 25.8%) dan ICU (n = 21; 23.6%). Majoriti pencilan dari swab luka (n = 33; 37%), T / aspirasi (n = 16; 18%) dan tisu (n = 10; 11.2%) mengandungi gen *QacE*.

Diikuti oleh ujian kerentanan antibiotik dan antiseptik, semua pencilan tertakluk kepada "finger printing" melalui pendekatan pulsed field gel electrophoresis (PFGE). Analisis menunjukkan tujuh kelompok (A-B-C-D-E-F dan G) dengan tahap kepelbagaian yang tinggi antara pencilan dan wad yang berbeza. Tahap rintangan untuk 25 antibiotik telah dibandingkan antara ketujuh-tujuh kelompok dan keputusan menunjukkan perbezaan yang signifikan bagi kumpulan (p = 0.019). Rintangan tertinggi diperhatikan dalam kelompok C (23,667 - carbapenems) manakala yang terendah milik kedua-dua kelompok B (22,000 - cephalosporins) dan F (22,000 - quinolones).

Corak rintangan antimikrob antara pencilan yang berbeza dengan rintangan yang sama dalam kelompok yang berbeza diperhatikan dalam tiga kelas antibiotik utama. *QacE*, *QXA58* dan *PER* mempunyai variasi yang tertinggi. Julat frekuensi untuk *QacE* adalah di antara 100% (kelompok B) dan 42.9% (kelompok F), dan julat ini untuk *OXA58* adalah di antara 94.4% (kelompok A) dan 28.6% (kelompok F), dan bagi *PER* adalah antara 100% (kelompok B) dan 66.7% (kelompok C). Dalam erti kata lain, ketiga-tiga gen mempunyai perubahan lebih tinggi di kalangan sampel yang telah membentuk kelompok yang berbeza ini.

Biofilm dan "quorum sensing" (QS) adalah dua fenomena yang sangat penting dalam membantu MDRAB untuk berpaut kepada kulit, mukosa, dan permukaan objek tidak bernyawa seperti peralatan perubatan (ventilator dan aspirasi trakea) dalam persekitaran hospital. Biofilm yang dikenali untuk meningkatkan rintangan dadah dalam bakteria. "quorum sensing" (QS) adalah ciri dalam bakteria yang membolehkan ia memantau kepadatan populasi melalui pengeluaran molekul isyarat kecil yang dikenali sebagai "auto inducers". Dalam MDRAB "auto inducers" adalah acyl-homoserine lactones (AHLs), (Chenia, 2013). Perkaitan yang kukuh antara gen rintangan antibiotik dan QS melalui isyarat laluan adalah kukuh. Dalam kajian ini,

pembentukan biofilm oleh pencilan MDRAB sangat resistan dipilih untuk dianalisis dan telah disaring untuk gen QS abaI. Pembentukan biofilm meningkat 2-4 kali ganda dalam MDRAB resistan dan adalah lebih tinggi berbanding dengan strain kawalan *Escherichia coli*.

Kesimpulannya, MDRAB biasa didapati di ICU, resistan kepada kebanyakan antibiotic yang digunakan untuk rawatan termasuk carbapenems dan membawa gen rintangan antibiotik. Walaupun kelaziman tinggi *QacE* gen yang bertanggungjawab untuk ketahanan antiseptik diperhatikan, MIC adalah lebih kurang daripada kepekatan yang digunakan di hospital. Kepelbagaian genom adalah tinggi di kalangan MDRAB yang digasingkan, bagaimanapun rintangan adalah sama. Kehadiran gen *abaI* dalam MDRAB yang menghasilkan kepekatan biofilm tinggi menunjukkan ia memerlukan perhatian lebih kerana kehadiran biofilm ini di persekitaran hospital dan permukaan pada objek tidak bernyawa seperti kateter, shunts dan ventilator. Pemantauan berkala untuk rintangan antibiotik dan antiseptik dalam wad adalah penting untuk pengurusan yang berkesan bagi MDRAB di hospital.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Vasantha Kumari Neela, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Rukman Awang Hamat, MPath
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Syafinaz Amin Nordin MBChB, MPath
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Anita Sulong, MPath
Medical Lecturer
Universiti Kebangsan Malaysia
Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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AST	Antibiotic susceptibility test
ATCC	American type culture collection
A.b	*Acinetobacter baumannii*
BLAST	Basic local alignment search tool
Bp	base pair
CFU	Colony forming unit
CLSI	Clinical and laboratory standard institute
DND	Deoxyribonucleic acid
E.coli	*Escherichia coli*
ICU	Intensive care unit
Kb	Kilo-base pair
LB	Luria-Bertani broth
MDR	Multi drug resistance
MDRAB	Multi drug resistance *Acinetobacter baumannii*
MHA	Muller Hinton agar
MHB	Muller Hinton broth
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
QAC	Quaternary ammonium compound
RNA	Ribonucleic acid
SE	Buffer consisting of mixture of Na-EDTA, NaCl, and distilled
Spp	Species

SPSS	Statistical package for the social sciences
TBE	Mixture of tris base, boric acid, EDTA and water
TE	Tris borate-ethylene diamine tetra acetic acid
V	Voltage
Mg	Microgram
µL	Microliter

CHAPTER 1

INTRODUCTION

Multiple drug resistant *Acinetobacter baumannii* (MDRAB) is recognized as one of the top nosocomial pathogens because of its environmental elasticity, potency to colonize several sites of body in hospitalized patients, prolonged permanence, association with MDR successful and isolates outbreak potential (Villegas *et al.,* 2003; Safdar *et al.,* 2006; Le *et al.,* 2007 ; Manian *et al.,* 2011 and Cockerill *et al.,* 2012). Although *A. baumannii* is considered as an organism of limited virulence, this microbe is of great clinical importance especially among individuals with weak immune systems, chronic lung disease, patients on ventilators or invasive devices like urinary catheters, prolonged hospital stay and also on those with open wounds.

It leads to an extensive spectrum of hospital acquired infections (HAI) including bloodstream infections, respiratory tract and UTI, ventilator related pneumonia (VAP), normally among ICU (Intensive Care Unit) patients. (Bergogne *et al*., 1996 and Perez *et al.,* 2007). The major transmission sources of *A. baumannii* infections are through person to person contacts, contaminated surfaces (Otter *et al.,* 2011) and room so occupied by previous patients diagnosed with *A. baumannii* colonization orinfection. Epidemiological investigations have obviously revealed that colonized patients and hospital environment are the main source of *A. baumannii* infections (Mak *et al*., 2009). *A. baumannii* infections Management is the very challenging forinfection, clinicians and patients control personnels. Interventions of infection control including hand hygiene compliance, patient screening, team isolation, environmental contamination supervision, increase environmental disinfection and cleaning have been revealed to decrease nosocomial infection degrees and outbreaks (McDonnell *et al*., 1999; Villegas *et al*., 2003 and Rodriguez *et al*., 2009).

As a definition, MDRAB is resistant to more than three classes of antibiotics including all penicillins and cephalosporins (inhibitor combinations), fluoroquinolones and aminoglycosides. Four categories of antibiotic resistance exist in *A. baumannii*, which include 1- produces enzymes that inactivate antimicrobials, 2- reduce access of antibiotics to targets due to low outer membrane permeability, 3- multidrug efflux pump and 4- mutations that alter cellular functions.

Certain strains of *A. baumannii* is highly resistant to most antibiotics available in clinical practice. A number of resistance mechanisms to many classes of antibiotics are known to exist in *A. baumannii*, including β-lactamases, multidrug efflux pumps, aminoglycoside-modifying enzymes, permeability defects, and the alteration of target sites. Most of these resistance mechanisms can target different classes of antibiotics. However, several different mechanisms can work together to contribute to the resistance to a single class of antibiotics. For example, the resistance mechanisms in CRAB are diverse. In addition to β-lactamases with carbapenem-hydrolyzing activity as a major carbapenem resistance mechanism, which include carbapenem-hydrolyzing

class D β-lactamases (CHDLs) and metallo-β-lactamases (MBLs), Penicillin-Binding Protein modifications might also be involved in carbapenem resistance. The spread of multidrug-resistance determinants in *A. baumannii* is mostly through plasmid conjugation, transposon acquisition or integron mobilization to gain clusters of genes encoding resistance to several antibiotic families (aminoglycosides). Modifications to DNA gyrase or topoisomerase IV through mutations in the *gyr*A and *par*C (quinolones), resistance mediated by efflux or ribosomal protection (tetracycline and glycylcyclines) (Ming *et al.,* 2014).

In Malaysia, detailed information on the antimicrobial resistance and genetic relationship of *A. baumannii* strains is still lacking (Boon Hong *et al*., 2011). Molecular epidemiology studies in Malaysia can offer a better awarness of *A. baumannii* epidemiology in the hospital and the likely transmission routes. Information of alterations in the *A. baumannii* rates resistance in hospitals can expand antimicrobial treatment.

To manage the increasing prevalence and control of life threatening infections caused by MDRAB, proper and timely identification, antibiotic resistance patterns and strain relatedness with epidemiology need to be well understood. This can aid in formulating new infection control policies, and reducing the mortality and morbidity rates. Understanding the type of MDR resistance has important concepts in infection control diseases and prevention, establishing antimicrobial stewardship programs in the community and hospital care (Carriço *et al*., 2013).

Antiseptics play a crucial role in the infection control trials and are adopted in skin antisepsis, and preparation of anatomical locations for surgical operations, hands sterilization prior contacting to an infected patient, before an intensive process and for a surgical scrub. Using antiseptics frequently in hospital has enhanced worries on its resistance. Recently a reserch by Suwantarat (2014) has reavealed that bacteria which causes life threatening infections in drastically ill patients are becoming less susceptible to the antiseptics which are used frequently in the hospitals. Patients who received common antiseptics wash observed low susceptibility to CLX in comparison with patients who did not receive the antiseptic baths.

Resistance to antiseptic is determined by the *qac* genes. At present numerous *qac* genes including *qac A/B* genes (McGann *et al*., 2011), *qac C/D* also known as *smr* (Bischoff *et al*., 2012), *qacE/F* (Ploy *et al*., 1998), *qacG* (Heir *et al*., 1999), *qacH* (Heir et *al*., 1998), *qac J* (Bjorland *et al*., 2003)and *qac Z* (Braga *et al.,* 2011) have been stated *Qac A/B* together with *qac C/D* genes is commonly related to Gram positive (Zmantar*et al.,* 2012; Longtin *et al.,* 2011), while *qac E* is regularly observed in Gram negative bacteria. Studies in Asia countries such as Malaysia and Singapore have revealed persistent growth of extremely MDR *A. baumannii* in Asia (Chung *et al.,* 2011). Correlation between reduced susceptibility to disinfectants and multidrug resistance among clinical isolates of *Acinetobacter* species has also been demonstrated (Sato *et al.,* 2010).

To control the spread of *A. baumannii* in the hospital, it is necessary to identify potential reservoirs of the organism and the modes of transmission. To distinguish the outbreak strain from epidemiologically unrelated ones, a comparison of isolates at the subspecies level is required. Molecular epidemiologic investigations are important to determine whether a clonal outbreak strain is present in the setting, and to trace the source of outbreak and route of transmission.

Biofilms and quorum sensing (QS) are two phenomena that are very important and crucial in helping the MDRAB to cling to the skin, mucosal, and surfaces of inanimate objects such as medical devices (ventilator and tracheal aspirate) in hospital environments. Most importantly, biofilms can increase the drug resistance of the strains. Bacteria producing biofilms are far more resistant to antimicrobial agents than those that do not produce (Gurung *et al*., 2013). QS as a shared system in bacteria allows the bacteria to monitor their population density through the production and sensing of small signal molecules known as auto inducers. In MDRAB auto inducers are acyl-homoserine lactones (AHLs), (Chenia, 2013). The strong association between antibiotic resistance genes and QS through signalling pathway has been well established. It is reported that more than 60% of the nosocomial infections in hospitals worldwide are due to biofilm formations on medical devices (M'hamedi *et al.,* 2014).

It is difficult to control MDRAB especially in intensive care units and to treat due to the limited antibiotic choices. The effective control approaches include isolation of patient and strict hygiene practices. In Malaysia, although MDR *A. baumannii* is one of the important nosocomial pathogen, its susceptibility towards antibiotics and antiseptics is not very well established. Therefore, this study is designed to understand the antibiotic and antiseptic susceptibility, molecular epidemiology and quorum sensing property of MDRAB collected from Universiti Kebangsaan Malaysia Medical Centre (UKMMC) that is one of the major Malaysian teaching hospitals. The study hospital was carried out in UKMMC, as it receives population from all over Klang valley and also has shown enhancing trends of MDRAB infections.

Research objective

General objectives

To investigate the molecular epidemiology of multiple drug resistant *A. baumannii* (MDRAB) in a tertiary care hospital in Malaysia.

Specific objectives

1. To determine the prevalence of MDRAB among the inpatients at study hospital.
2. To determine the antimicrobial susceptibility and resistance gene patterns of MDRAB.
3. To determine the antiseptic susceptibility and resistance genes in MDRAB.
4. To determine the molecular epidemiology of MDRAB in the study hospital.

5. To investigate the association between quorum sensing and antibiotic resistance in MDRAB isolates.

REFERENCES

Abbott, I, Cerqueira, GM., Bhuiyan, S., Peleg, AY. (2013). Carbapenem resistance in *Acinetobacter baumannii*: laboratory challenges, mechanistic insights and therapeutic strategies. *Expert Rev Anti Infect Ther*, 11(4),395- 409.

Adams,H., (2008). Genetic basis of multidrug resistance in *Acinetobacter baumannii* clinical isolates at a tertiary medical center in Pennsylvania. *Antimicrob Agents Chemother*, 52(11),3837-43.

Adams,H., (2011). Molecular epidemiology of carbapenem-nonsusceptible *Acinetobacter baumannii* in the United States. *Journal Clin Microbiol*,49(11),3849-54.

Afreenish, H., Javaid U., Fatima, K., Aslam, K., Zakir, H. (2010). In vitro activity of aminoglycosides, β-lactam-β-lactamases inhibitor combinations and tetracyclines against multi-drug resistant *Acinetobacter baumannii*, isolated from a tertiary care hospital. *J Microbiol Antimicrob*, 2(4), 47–50.

Al-Anazi, KA., Abdulhamid, B., Alshibani, Z., Awad, K., Alzayed, A., Hassan, H. (2012). *Acinetobacter baumannii* septicemia in a recipient of an allogeneic hematopoietic stem cell transplantation. *Hindawi Pub Corpor*, 20(12), 1-5.

Richard, D., Sherry, S.(2004). Amplification of ribosomal RNA sequences. *Molecular Microbial Ecology Manual*, 3.01, 509–522.

Anbazhagan, D., Mansor, M., Yan, G. (2012). Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of *Acinetobacter spp*. *PloS One,* 7(7).

Anunnatsiri, S., Tonsawan, P. (2011). Risk factors and clinical outcomes of multidrug-resistant *Acinetobacter baumannii* bacteremia at a university hospital in Thailand. *Southeast Asian J Trop Med Public Health*,42(3),693-703.

Arvaniti, K., Lathyris, D., Ruimy, R. (2012). The importance of colonization pressure in multiresistant *Acinetobacter baumannii* acquisition in a Greek intensive care unit. *Crit Care,* 13,16(3).

Bae, I., Jeong, S., Lee, K. (2012). Carbapenem-Resistant *Acinetobacter baumannii*. *Korean J of Clin Microbiol*,15(1),1-8.

B. A, Evans., S. Amyes. (2014). *OXA* β-Lactamases. *Clin. Microbiol. Rev*, 27 2,241-263.

Bartual, S., Seifert, H. (2005). Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii. J Clin Microbiol,*43(9),4382-90.

Bassetti, M., Righi, E., Esposito, S., Petrosillo, N., Nicolini, L. (2008). Drug treatment for multidrug-resistant *Acinetobacter baumannii* infections. *Future Microbiol*, 3(6),649-60.

Baumann, U., Mansouri, E., Specht, B. (2004). Recombinant OprF–OprI as a vaccine against *Pseudomonas aeruginosa* infections. *Vaccine*,17,22(7),840-7.

Bjorland, J., Steinum, T., Sunde, M., Waage, S., Heir, E. (2003).Novel plasmid-borne gene *qacJ* mediates resistance to quaternary ammonium compounds in equine *Staphylococcus aureus, Staphylococcus simulans,* and *Staphylococcus intermedius*. *Antimicrobial Agents and Chemoter*, 47, 3046–3052.

Bischoff, M., Bauer, J., Preikschat, P., Schwaiger, K., Molle, G.,Holzel, C. (2012). First detection of the antiseptic resistance gene *qacA/B* in *Enterococcus faecalis*. *Microbiol Drug Res*is,18, 7–12.

Boddey, J., Flegg, C., Day, C. (2006). Temperature-regulated microcolony formation by *Burkholderia pseudomallei* requires pilA and enhances association with cultured human cells. *Infect Immun,* 74(9), 5374–5381.

Bonomo, R., Szabo, D. (2006). Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infec Dis*, 43, 49-56.

Braga, TM., Marujo, PE., Pomba, C., Lopes, MFS. (2011).Involvement, and dissemination, of the enterococcal small multidrug resistance transporter *QacZ* in resistance to quaternary ammonium compounds. *J of Antimicrob Chemoter*, 66, 283–286.

Cai, Y., Wang, R., Liang, B., Bai, N., Liu, Y. (2011). Systematic review and meta-analysis of the effectiveness and safety of tigecycline for treatment of infectious disease.*Antimicrob Agents Chemother* , 55(3),1162–72.

Canduela, J., Gallego, L., Sevillano, E., Valderrey, C., Calvo, F., Pérez, J. (2006). Evolution of multidrug-resistant *Acinetobacter baumannii* isolates obtained from elderly patients with respiratory tract infections. *The J of Antimicrob Chemoter*, *57*, 1220–1222.

Carriço, J. a, Sabat, a J., Friedrich, a W., Ramirez, M. (2013). Bioinformatics in bacterial molecular epidemiology and public health: databases, tools and the next-generation sequencing revolution. *Euro Surveill*, 24;18(4),20382.

Chang, Y., Shih, D., Wang, J., Yang, S. (2007). characterization of class 1 integrons and antimicrobial resistance in *Aeromonas* strains from foodborne outbreak-suspect samples and environmental sources. *Diagn Microbiol Infect Dis*, 59(2),191-7.

Chastre, J. (2003). Infections due to *Acinetobacter baumannii* in the ICU. *Seminars in Resp and Cri Care Med*, 24(1),69-78.

Chawla, R. (2008). Epidemiology, etiology, and diagnosis of hospital-acquired pneumonia and ventilator-associated pneumonia in Asian countries. *American J of Infec Control*, 36(4 Suppl),S93-100.

Chelikani, P., Fita, I., Loewen, P. (2004). Diversity of structures and properties among catalases. *Cell Mol Life Sci*, 61(2),192-208.

Chen, Q., Li, X., Zhou, H. (2014). Decreased susceptibility to tigecycline in *Acinetobacter baumannii* mediated by a mutation in trm encoding SAM-dependent methyltransferase. *J Antimicrob Chemother,* 69(1),72-6.

Chenia, H. (2013). Anti-quorum sensing potential of crude Kigelia africana fruit extracts. *Sensors (Basel, Switzerland)*, *13*(3), 2802–17.

Chopra, S., Galande, A. (2011). A fluoroquinolone-resistant *Acinetobacter baumannii* without the quinolone resistance-determining region mutations. *J of Antimicrob,* 66(11),2668-70.

Chung, DR., Song, JH., Kim, SH., Huang, SG.,Wang, H., So, TM., Yasin, RM., Hsueh, PR., Carlos, CC., Hsu, LY., Buntaran, L., Lalitha, MK., Kim, MJ., Choi, JY., Kim, SI., Ko, KS., Kang, CI., Peck, KR. (2011). High prevalence of multidrug-resistant non-fermenters in hospital-acquired pneumonia in Asia. *Am. J. Respir. Crit. Care Med,* 184,1409–1417.

Cisneros, J., Rodriguez-Bano, J. (2002). Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. *Clin Microbiol Infect*, 8(11),:687-93.

C. Landelle, P., Legrand, P., Lesprit . (2013). "Protracted outbreak of multidrug-resistant *Acinetobacter baumannii* after intercontinental transfer of colonized patients," *Infec Control and Hosp Epidemiol*, 34 (2), 119–124.

Cockerill, F. (2012). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement. *Clinical and Laboratory Standards Institute,32(3),30-43.*

Constantiniu, S., Romaniuc, A., Luminiţa Smaranda Iancu, F., Taraşi, I. (2004). Cultural and biochemical characteristics of *acinetobacter spp* . Strains isolated from hospital units. *The J of Preven Medic*, *12*, 35–42.

Corbella, X., Montero, A., Pujol, M . (2000). Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multiresistant *Acinetobacter baumannii* . *J Clin Microbiol* , 38, 4086–4095.

Crespo, M., Woodford, N. (2004). Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-8, a novel metallo-β-lactamase, in a tertiary care center in Cali, Colombia. *J Clin Microbiol*, 42(11), 5094–5101.

Cunha, B. (2010). Optimal therapy for multidrug-resistant *Acinetobacter baumannii*. *Emerg Infec Dis*, 16(1),170.

Dai, N., Li, D., Chen, J., Chen, Y., Geng, R. (2010). Drug-resistant genes carried by *Acinetobacter baumanii* isolated from patients with lower respiratory tract infection. *Chin Med J (Engl)*,123(18),2571-5.

Dent, L., Marshall, R., Pratap, S., Hulette, B. (2010). Multidrug resistant *Acinetobacter baumannii*: a descriptive study in a city hospital. *BMC Infec Dis*, 10,196.

D. J., Morgan, Stephen, Y. L, Catherine, L. S. (2010) Frequent Multidrug-Resistant *Acinetobacter baumannii* Contamination of Gloves, Gowns, and Hands of Healthcare Workers. *Infect Control Hosp Epidemiol*, 31(7), 716–721.

D. McDougald, S, Rice., S, Kjelleberg. (2007). Bacterial quorum sensing and interference by naturally occurring biomimics. *Anal Bioanal Chem*, 387, 445–453.

Durmaz, R., Otlu, B., Koksal, F., Hosoglu, S. (2009). The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella spp*. *Jpn J Infect Dis*, 62(5),372-7.

E Bergogne, B., K, J,. Towener. (1996): *Acinetobacter spp.* as nosocomial pathogens: microbiological, clinical and epidemiological features. *Clin Microbiol Rev*, 9, 148–165.

Erol, S.Altoparlak, U,Akcay, MN, Celebi, F, Parlak, M.(2004). Changes of microbial flora and wound colonization in burned patients. *Burns*,30(4),357-61.

Faddin, J. (1981). Biochemical tests for identification of medical bacteria. *J Clin Pathol*, 34(5), 572.

Falagas, E., Karveli, A. (2007). The changing global epidemiology of *Acinetobacter baumannii* infections: A development with major public health implications. *Clin Microbiol and Infec*, *13*, 117–119.

Fallah, F., Noori, M., Hashemi, A., Karimi, A. (2014). Prevalence of *bla NDM, bla PER, bla VEB, bla IMP* and *bla VIM* type genes among *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran, *Scientifica (Cairo).* 2014:245162.

F A, Manian.,S, Griesenauer., D, Senkel., J, M Setzer., S ,A Doll. (2011). Isolation of *Acinetobacter baumannii* complex and methicillin-resistant *Staphylococcus aureus* from hospital rooms following terminal cleaning and disinfection: can we do better? *Infect Control Hosp Epidemiol*, 32, 667–672.

Forbes, B., Sahm, D., Weissfeld, A. (1998). Bailey & Scott's diagnostic microbiology. Retrieved from http://www.elsevierhealth.com/us/product/toc.jsp?isbn=9780323030656

F, Perez., A, M. Hujer., K M, Hujer., B K, Decker., P N, Rather., R A, Bonomo (2007). Global challenge of multidrug-resistant *Acinetobacter baumannii* .*Antimicrobiol Agents Chemother*, 51,3471–3484.

Gabriel, P. S., Zhou, J., Tabibi, S. (2004). Antimicrobial susceptibility and synergy studies of *Stenotrophomonas maltophilia* isolates from patients with cystic fibrosis. *Antimicrobiol Agent* , vol. 48, no. 1, 168-171.

Gaddy, J., Actis, L. (2009). Regulation of *Acinetobacter baumannii* biofilm formation. *Future Microbiol*, 4(3),273-8.

Gales, AC., Jones, RN., Forward, KR., Linaris, J., Sader, HS., Verhoef, J. (2001). Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas* maltophilia as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY antimicrobial surveillance program (1997-1999). *Clin Infect Dis,* 32(Suppl 2),104–13.

Gaze, W., Abdouslam, N. (2005). Incidence of class 1 integrons in a quaternary ammonium compound-polluted environment. *Antimicrobiol Agent,* 49(5), 1802–1807.

George, M. E., Lisa, L. M., Trish, M. P. (2008). *Acinetobacter baumannii*: Epidemiology, Antimicrobial Resistance, and Treatment Options. *Clin Infect Dis* , 46 (8),1254-1263.

Gonçalves, C., Vaz, T. (2000). serotyping and ribotyping as epidemiological tools in the evaluation of *Acinetobacter baumannii* dissemination in hospital units, Sorocaba, Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo*,42(5),277-82.

González, R., Nusblat, A., Nudel, B. (2001). Detection and characterization of quorum sensing signal molecules in *Acinetobacter* strains. *Microbiol Research*, Volume 155, Issue 4,271–277.

Gurung, J., Khyriem, A. B., Banik, A., Lyngdoh, W. V., Choudhury, B., Bhattacharyya, P. (2013). Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from intensive care unit. *Indian J of Crit Care Medic* , *17*(4), 214–8.

Heir, E., Sundheim, G., Holck, AL. (1998). The *Staphylococcus qacH* gene product: a new member of the SMR family encoding multidrug resistance. *FEMS Microbiol Letters,* 163, 49–56.

Heir, E., Sundheim, G., Holck, AL. (1999). The *qacG* gene on plasmid pST94 confers resistance to quaternary ammonium compounds in *staphylococci* isolated from the food industry. *J of Applied Microbiol*, 86, 378–388.

Higgins, P. G., Dammhayn, C., Hackel, M., Seifert, H. (2009). Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J of Antimicrobiol Chemoter*, *65*, 233–238.

Ho, C.-M., Li, C.-Y., Ho, M.-W., Lin, C.-Y., Liu, S.-H., Lu, J.-J. (2012). High rate of *qacA*- and *qacB*-positive methicillin-resistant *Staphylococcus aureus* isolates from chlorhexidine-impregnated catheter-related bloodstream infections. *Antimicrobiol Agents and Chemoter*, *56*(11), 5693–7.

Horner, C., Mawer, D., Wilcox, M. (2012). Reduced susceptibility to chlorhexidine in *staphylococci*: is it increasing and does it matter? *The J of Antimicrobiol Chemoter*, *67*(11), 2547–59.

Howard, A., O'Donoghue, M., Feeney, A., D. Sleator, R. (2012). *Acinetobacter baumannii* An emerging opportunistic pathogen. *Virulence*, 1; 3(3), 243–250.

Huber, C., Sartor, A., McOdimba, F. (2014). Outbreaks of multidrug-resistant *Acinetobacter baumannii* strains in a Kenyan teaching hospital. *J of Global Antimicrobiol Resistance,* 2 (3), 190–193.

Hujer, K., Hujer, A. (2009). Rapid determination of quinolone resistance in *Acinetobacter spp*. *J. Clin. Microbiol*, 47 (5), 1436-1442.

J. A, Otter., S, Yezli., G L, French, (2011), The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect Control Hosp Epidemiol*, 32, 687–699.

Jean, L., Christine, S.,Krystal, S., Allison, M., Andrew, S.,Yves, L.,Donald, E., Roberto, G Melano. (2011).Distribution of Antiseptic Resistance Genes *qacA, qacB*, and *smr* in Methicillin-Resistant *Staphylococcus aureu*s Isolated in Toronto, Canada, from 2005 to 2009. *Antimicrobiol Agents and Chemoter* , 55,2999–3001.

Jeong, S., Bae, I., Park, K. (2006). Outbreaks of imipenem-resistant *Acinetobacter baumannii* producing carbapenemases in Korea. *J Microbiol*,44(4),423-31.

J Rodriguez,-Bano., L Garcia., E Ramirez., L Martinez-Martinez., MA. Muniain., F Fernandez. (2009). Long-term control of hospital-wide, endemic multidrug-resistant *Acinetobacter baumannii* through a comprehensive "bundle" approach. *Am J Infect Control*, 37:715–722.

Jung, J., Park, M., Kim, S. (2010). Risk factors for multi-drug resistant *Acinetobacter baumannii* bacteremia in patients with colonization in the intensive care unit. *BMC Infect Dis,* 30,10,228.

Kabbaj, H., Seffar, M., Belefquih, B. (2012). Prevalence of Metallo-β-Lactamases Producing *Acinetobacter baumannii* in a Moroccan Hospital. *ISRN Infec Dis*,2013,3.

Kawamura,S., K J, Wachino., T, Kondo., H, Ito., Y, Arakawa. (2010). Correlation between reduced susceptibility to disinfectants and multidrug resistance among clinical isolates of *Acinetobacter* species. *J. Antimicrobiol Chemother* , 65 ,1975–1983.

Kim, D., Choi, J., Kim, H. (2013). Spread of carbapenem-resistant *Acinetobacter baumannii* global clone 2 in Asia and *AbaR*-type resistance islands. *Antimicrobiol Agents Chemother*,57(11),5239-46.

Kohlenberg, A., Brümmer, S. (2009). Outbreak of carbapenem-resistant *Acinetobacter baumannii* carrying the carbapenemase *OXA-23* in a German university medical centre. *J of Medic Microbiol*, 58, 1499–1507.

Kong, B., Hanifah, YA., Yusof, MY., Thong, KL.(2011). Antimicrobial susceptibility profiling and genomic diversity of multidrug-resistant *Acinetobacter baumannii* isolates from a teaching hospital in Malaysia. *Jpn J Infect Dis*,64(4), 337-40.

Kuo, H., Yang, C., Lin, M., Cheng, W. (2010). Distribution of *bla OXA*-carrying imipenem-resistant *Acinetobacte*r *spp*. in 3 hospitals in Taiwan. *Diagno Microbiol Infect Dis*,66(2),195-9.

Lain, A., Gustavo, M., Saruar, B., Anton, Y. (2013). Carbapenem resistance in *Acinetobacter baumannii*: laboratory challenges, mechanistic insights and therapeutic strategies. *Exp Rev of Anti-infec Thera*, 11(4), 395-409.

Lean, S., Suhaili, Z., Ismail, S. (2014). Prevalence and Genetic Characterization of Carbapenem-and Polymyxin-Resistant *Acinetobacter baumannii* Isolated from a Tertiary Hospital in Terengganu,malaysia. *ISRN Microbiol*, 19,2014,953417.

Lee, H., Koh, Y., Kim, J., Lee, J. (2008). Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. *Clin Microbiol Infect,*14(1),49-54.

Lee, P., Costumbrado, J. (2011). Agarose gel electrophoresis for the separation of DNA fragments. *J Vis Exp*, 20,(62), pii, 3923.

Le, T., M, D., M, J Dibley., M D, Vo Van Nho., L, Archibald., W R, Jarvis., A H, Sohn. (2007). Reduction in surgical site infections in neurosurgical patients associated with a bedside hand hygiene program in Vietnam. *Infect. Control Hosp. Epidemiol* , 28 ,583–588.

Lee, Y., Bae, I., Kim, J. (2012). Dissemination of ceftazidime-resistant *Acinetobacter baumannii* clonal complex 92 in Korea. *J Appl Microbiol*,112(6),1207-11.

Lim, T., Tan, T., Lee, W., Sasikala, S., Tan, T. (2011). In-vitro activity of polymyxin B, rifampicin, tigecycline alone and in combination against carbapenem-resistant *Acinetobacter baumannii* in Singapore. *PLoS One*, 21,6(4),e18485.

Mardia, K. V., Kent, J. T., Bibby, J. M. (1979). *Multivariate analysis*. *Biometrical J.*

M'hamedi, I., Hassaine, H. (2014). Biofilm formation by *Acinetobacter baumannii* isolated from medical devices at the intensive care unit of the University Hospital of Tlemcen (Algeria). *Afri J of Microbiol Research*, Vol.8(3), pp, 270-276.

Mah, T., O'Toole, G. (2001). Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol*,9(1),34-9.

Mahzounieh, M., Khoshnood, S. (2014). Detection of Antiseptic-Resistance Genes in *Pseudomonas* and *Acinetobacter spp.* Isolated From Burn Patients. *Jundishapur J Nat Pharm Prod*, 3,9(2),e15402.

Mak ,JK., Kim, MJ., Pham, J., Tapsall, J., White, PA. (2009). Antibiotic resistance determinants in nosocomial strains of multidrug-resistant *Acinetobacter baumannii. J of Antimicrobiol Chemoter*,63, 47–54.

Manchanda, V., Sanchaita, S., Singh, N. (2010). Multidrug resistant acinetobacter. *J of Global Infec Dis*, *2*(3), 291–304.

Manian, FA., Griesenauer, S., Senkel, D., Setzer, JM., Doll, SA., Perry, AM. (2011). Isolation of *Acinetobacter baumannii* complex and methicillin-resistant *Staphylococcus aureus* from hospital rooms following terminal cleaning and disinfection: can we do better? I*nfect Control Hosp Epidemiol*,32,667–72.

Maragakis, L., Perl, M. (2008). *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options. *Clin Infec Dis :46*, 1254–1263.

Marque, S., Poirel, L., He´ritier, C. (2005). Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacillinase *OXA-58* in *Acinetobacter spp*. in Europe. *J Clin Microbiol* , 43,4885–4888.

McDonnell, G., Russell, AD. (1999). Antiseptics and disinfectants: Activity, action, and resistance. *Clin Microb Rev*, 12, 147–179.

McGann, P., Kwak, YI., Summers, A., Cummings, JF., Waterman, PE., Lesho, EP. (2011). Detection of *qacA/B* in clinical isolates of methicillin-resistant *Staphylococcus aureus* from a regional healthcare network in the eastern United States. *Infec Control and Hosp Epidemiol*, 32, 1116–1119.

M. Denton, M. H. Wilcox, P. Parnell . (2004). "Role of environmental cleaning in controlling an outbreak of *Acinetobacter baumannii* on a neurosurgical intensive care unit." *J of Hosp Infec*, 56, (2), 106–110.

M. E. Falagas ,P. Kopterides. (2006). "Risk factors for the isolation of multi-drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature," *J of Hosp Infec*, 64,(1), 7–15.

Mezzatesta, M. (2012). Epidemiological characterization and distribution of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Italy. *Clin Microbiol Infect,*18(2),160-6.

Milstone, A. M., Passaretti, C. L., Perl, T. M. (2008). Chlorhexidine: expanding the armamentarium for infection control and prevention. *Clin Infec Dis 46*(2), 274–81.

Ming, F. L., Chung,Y. L. (2014). Antimicrobial resistance in *Acinetobacter baumannii*: From bench to bedside. *World J Clin Cases*, 2(12), 787–814.

Mohajeri, P., Farahani, A., Feizabadi, M. M., Ketabi, H., Abiri, R., Najafi, F. (2013). Antimicrobial susceptibility profiling and genomic diversity of *Acinetobacter baumannii* isolates: A study in western Iran. *Iranian J of Microbiol*,*5*,195–202.

Mohammadreza, M., Sheida, Kh., Azizollah, E., Saeid, H.,Maryam, Y. (2014). Detection of Antiseptic-Resistance Genes in *Pseudomonas* and *Acinetobacter spp*. Isolated From Burn Patients. *Jundishapur J Nat Pharm Prod*, 9(2), e15402.

Moriel, D. G., Beatson, S. A., Wurpel, D. J., Lipman, J., Nimmo, G. R., Paterson, D. L., Schembri, M. A. (2013). Identification of Novel Vaccine Candidates against Multidrug-Resistant *Acinetobacter baumannii*. *PLoS ONE*, 8(10),e77631.

Morovat, T., Bahram, F. (2009). Distribution of different carbapenem resistant clones of *Acinetobacter baumannii* in Tehran hospitals. *New Microbiol*,32(3),265-71.

M T, gillespie., B R, lyon., L J, messerolti., R, A skurray. (1987). Chromosome- and plasmid-mediated gentamicin resistance in *Staphylococcus aureus* encoded by Tn4007 *J. Med. Microbiol* , 24, 139-144.

Munoz-Price, S. (2010). Clinical Outcomes of Carbapenem-Resistant *Acinetobacter baumannii* Bloodstream Infections: Study of a 2-State Monoclonal Outbreak. *Infect Control Hosp Epidemiol,*31(10),1057-62.

M V, Villegas., A I, Hartstein. (2003).*Acinetobacter* outbreaks, 1977–2000. *Infect Control Hosp Epidemio*l, 24, 284–295.

Naas, T., Bogaerts, P., Bauraing, C., Degheldre, Y., Glupczynski, Y., Nordmann, P. (2006). Emergence of *PER* and *VEB* extended-spectrum beta-lactamases in *Acinetobacter baumannii* in Belgium. *The J of Antimicrobiol Chemoter*, *58*(1), 178–82.

Niu, C., Clemmer, K. (2008). Isolation and characterization of an autoinducer synthase from *Acinetobacter baumannii*. *J Bacteriol*, 190(9), 3386–3392.

Nidhi, B, Prince, SH., Neena, C. (2010). Quorum sensing in *Acinetobacter*: an emerging pathogen. *Critical Rev in Microbiol*, Volume 36- Issue 4.

Nowroozi, J., Sepahi, A. (2014). Evaluation of Ciprofloxacin (*gyrA, parC* Genes) and Tetracycline (*tetB* Gene) Resistance in Nosocomial *Acinetobacter baumannii* Infections. *Jundishapur J Microbiol*, 7(2), e8976.

N, Safdar., J, Marx., N A, Meyer., D G, Maki. (2006). Effectiveness of preemptive barrier precautions in controlling nosocomial colonization and infection by methicillin-resistant *Staphylococcus aureus* in a burn unit. *Am J Infect Control,* 34, 476–483.

Nseir, S., Blazejewski, C., Lubret, R., Wallet, F., Courcol, R., Durocher, A. (2011). Risk of acquiring multidrug-resistant Gram-negative bacilli from prior room occupants in the intensive care unit. *Clin Microbiol and Infec*, *17*, 1201–1208.

Nuntra, S., Carroll, KC., Tsigereda, T., Tracy, R., Maragakis, LL., Sara, C. (2014). "High Prevalence of Reduced Chlorhexidine Susceptibility in Organisms Causing Central Line-Associated Bloodstream Infections". *Infect Control Hosp Epidemiol*,35,1183–6.

Peleg, A., Franklin, C., Bell, J., Spelman, D. (2006). Emergence of carbapenem resistance in *Acinetobacter baumannii* recovered from blood cultures in Australia. *Infec Control and Hosp Epidemiol ,* 27(7), 759-761.

Peleg, A. Y., Seifert, H., Paterson, D. L. (2008). *Acinetobacter baumannii:* emergence of a successful pathogen. *Clin Microbiol Rev*, *21*, 538–582.

Perez, F., Hujer, A., Hujer, K. (2007). Global challenge of multidrug-resistant *Acinetobacter baumannii. Antimicrob. Agents Chemother*, 51 , 103471-3484.

Playford, EG, Craig, JC, Iredell, JR.. (2007). Carbapenemresistant *Acinetobacter baumannii* in intensive care unit patients: risk factors for acquisition, infection and their consequences. *J Hosp Infect*, 65(3), 204-11.

P.L, Hoa., A.Y, Hoa., K.H, Chowa., E.L, Laia., P. Chingb., W.H. Seto. (2010). Epidemiology and clonality of multidrug-resistant *Acinetobacter baumannii* from a healthcare region in Hong Kong. *J of Hosp Infec,* 74, 4, 358–364.

Ploy, MC., Courvalin, P., Lambert, T. (1998). Characterization of In40 of *Enterobacter aerogenes* BM2688, a class 1 integron with two new gene cassettes, *cmlA2* and *qacF*. *Antimicrobiol Agents and Chemoter* ,42, 2557–2563.

Potron, A., Munoz-Price, L. (2011). Genetic features of *CTX-M-15*-producing *Acinetobacter baumannii* from Haiti. *Antimicrobiol Agents Chemother*, 55(12), 5946–5948.

Pour, N., Dusane, D. (2011). Biofilm formation by *Acinetobacter baumannii* strains isolated from urinary tract infection and urinary catheters. *FEMS Immunol Med Microbiol,*62(3),328-38.

Pournaras, S., Markogiannakis, A., Ikonomidis, A. (2006). Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing *OXA-58* carbapenemase in an intensive care unit. *J Antimicrobiol Chemother*, 57, 557–561.

Quale, J., Bratu, S., Landman, D., Heddurshetti, R. (2003). Molecular epidemiology and mechanisms of carbapenem resistance in *Acinetobacter baumannii* endemic in New York City. *Clin Infect Dis*, 37, 214-20.

Queenan, A., Bush, K. (2007). Carbapenemases: the versatile β-lactamases. *Clin Microbiol Rev,*20(3),440-58.

Queipo, OM. (2008). Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. *Clin Vaccine Immunol*,15(2),293-6.

Rao, R., Karthika, R., Singh, S. (2008). Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of *Acinetobacter baumannii. Indian J Med Microbiol*, 26(4),333-7.

Rebecca, H., Sunenshine, Marc-Oliver, W., Lisa, L., Maragakis. (2007). Multidrug-resistant *Acinetobacter* Infection Mortality Rate and Length of Hospitalization. *Emerg Infec Dis* , Vol. 13, No. 1.

Robert, A., Bonomo, Dora, S. (2006). Mechanisms of Multidrug Resistance in *Acinetobacter* Species and *Pseudomonas aeruginosa*. *Clin Infec Dis*, 43, S49–56.

Rodriguez, J., Cisneros JM., Fernandez, F. (2004). Clinical features and epidemiology of *Acinetobacter baumannii* colonization and infection in Spanish hospitals. *Infect Control Hosp Epidemiol* , 25, 819–824.

Rossolini, GM., Mantengoli, E. (2008). Antimicrobial resistance in Europe and its potential impact on empirical therapy. *Clin Microbiol Infect,* 14(6),2–8.

R, Zarrilli, Crispino, M., Bagattini, M. (2004). Molecular epidemiology of sequential outbreaks of *Acinetobacter baumannii* in an intensive care unit shows the emergence of carbapenem resistance. *J Clin Microbial*, 42, 946-53.

Sengstock, D. M., Thyagarajan, R., Apalara, J., Mira, A., Chopra, T., Kaye, K. S. (2010). Multidrug-resistant *Acinetobacter baumannii*: an emerging pathogen among older adults in community hospitals and nursing homes. *Clin Infec Dis* , *50*, 1611–1616.

Shahcheraghi, F. (2011). genetic characterization of metallo-β-lactamase and carbapenamase producing strains of *Acinetobacter baumannii* from patients at Tehran hospitals. *Iran J Microbiol*, 3(2), 68–74.

Shahida, S. M., Islam, A., Dey, B., Islam, F., Venkatesh, K., Goodman, A. (2016). Hospital Acquired Infections in Low and Middle Income Countries: Root Cause Analysis and the Development of Infection Control Practices in Bangladesh. *Open J of Obstetr and Gynecol*, 6, 28-39.

Sheldon, A. (2005). Antiseptic "resistance": real or perceived threat? *Clin Infec Dis*, 40 (11),1650-1656.

Singh, A., Goering, R., & Simjee, S. (2006). Application of molecular techniques to the study of hospital infection. *Clin Microbiol Rev*,19(3),512-30.

Singh, H., Thangaraj, P., Chakrabarti, A. (2013). *Acinetobacter baumannii*: A Brief Account of Mechanisms of Multidrug Resistance and Current and Future Therapeutic Management. *J of Clin and Diagno Research : JCDR*, *7*, 2602–5.

Smith, M. G., Gianoulis, T. A., Pukatzki, S., Mekalanos, J. J., Ornston, L. N., Gerstein, M., Snyder, M. (2007). New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. *Genes Dev*, 1;21(5),601-14.

Sotgiu, G. (2014). Outbreak of multidrug-resistant *Acinetobacter baumannii* in an intensive care unit. *New Microbiol*. 37, 185-191.

Sunenshine, R. H., Wright, M.-O., Maragakis, L. L., Harris, A. D., Song, X., Hebden, J., Srinivasan, A. (2007). Multidrug-resistant *Acinetobacter* infection mortality rate and length of hospitalization. *Emerg Infec Dis*, *13*(1), 97–103.

Sung, J., Kwon, K., Park, J. (2008). Dissemination of *IMP-1* and *OXA type* β-lactamase in carbapenem-resistant *Acinetobacter baumannii*. *Korean J Lab Med,*28(1),16-23.

Talbot, G., Bradley, J. (2006). Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin Infect Dis*, 1;42(5),657-68.

Tarek, Z., Bochra, K., Hajer, H., Amina., B. (2012). Detection of disinfectant and antibiotic resistance genes in *Staphylococcus aureus* isolated from the oral cavity of Tunisian. *children Annals of Microbiol*, 62, 123-128.

Teo, j., peng, l, yang, h.l., yen, t.t., sasikala, s., yun, h.p., l kwa, p, apisarnthanarak, a. (2015). extensively drug-resistant *acinetobacter baumannii* in a thai hospital: a molecular epidemiologic analysis. *Antimicrobiol Resist and Infec Control,*4,2.

Thapa, B, Tribuddhar,at, C. (2010). High prevalence of bla *(OXA)-23* in oligoclonal carbapenem-resistant *Acinetobacter baumannii* from Siriraj Hospital, Mahidol University, Bangkok, Thailand. *Southeast Asian J Trop Med Public Health*,41(3),625-35.

Thomas, L., Russell, a D., Maillard, J.-Y. (2005). Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against *Pseudomonas aeruginosa* and its response to biocide residues. *J of Applied Microbiol*, *98*(3), 533–43.

Thong, K., Lai, M., Teh, C., Chua, K. (2011). of methicillin-resistant *Staphylococcusaureus, Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by multiplex PCR. *Tropic Biomedicine*. 28(1),21-31.

Turton, JF., Kaufmann, ME., Warner, M. (2004). A prevalent, multiresistant clone of *Acinetobacter baumannii* in Southeast England. *J Hosp Infect*, 58, 170–179.

Vila, J., Ruiz, J., Goni, P., Jimenez, T. (1997). Quinolone-resistance mutations in the topoisomerase IV *parC* gene of *Acinetobacter baumannii. J Antimicrobiol Chemoter*, 39, 757-62.

Villegas, M., Hartstein, A. (2003). *Acinetobacter* Outbreaks, 1977-2000. *Infec Control and Hosp Epidemiol*, 24, (4), 284-295.

Vipin, C. (2013). Quorum sensing inhibitors: An overview. *Biotech Advances*, 31, 2, 224–245.

Wang, X., & Wood, T. (2011). Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. *Appl and Environment Microbiol*. 77(16),5577-83.

Wiegand, I., Hilpert, K., Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, *3*, 163–175.

Williams, J., Halvorsen, E. (2011). Toxin–antitoxin (TA) systems are prevalent and transcribed in clinical isolates of *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett*,322(1),41-50.

Wisplinghoff, H., Seifert, H., Tallent, S. M., Bischoff, T., Wenzel, R. P., Edmond, M. B. (2003). Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features and susceptibilities. *Pediatr Infect Dis J*, *22*, 686–691.

Yadegarinia, D., Abedy, S. (2013). Prevalence and Drug Resistance of *Acinetobacter baumannii* in ICU of a Teaching Hospital. *J. Appl. Environ. Biol,* 3(9)22-27.

Yahav, D., Lador, A., Paul, M., Leibovici, L. (2011). Efficacy and safety of tigecycline: a systematic review and meta-analysis. *J Antimicrobiol Chemoter,* 66,1963–71.

Yangsoon, L., Jinhwa, L., Seok Hoon, J., Jonghan, L., Il Kwon, B., Kyungwon, L. (2010). Carbapenem-non-susceptible Acinetobacter baumannii of sequence type 92 or its single-locus variants with a G428T substitution in zone 2 of the rpoB gene. *J Antimicrobiol Chemother*, 66 (1), 66-72.

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., Walsh, T. R. (2009). Characterization of a new metallo-beta-lactamase gene, *bla(NDM-1)*, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrobiol Agents and Chemother*, *53*(12), 5046–54.

Yum, J., Yi, K., Lee, H., Yong, D. (2002). Molecular characterization of metallo-b-lactamase-producing *Acinetobacter baumannii* and *Acinetobacter* genomospecies 3 from Korea: identification of two new. *J Antimicrobiol Chemother,*49(5),837-40.

LIST OF PUBLICATIONS

Babaei, M.R., Sulong, A., Awang Hamat, R., Nordin, S, A., Neela, V. (2015). Extremely high prevalence of antiseptic resistant Quaternary Ammonium Compound E gene among clinical isolates of multiple drug resistant *Acinetobacter baumannii* in Malaysia. *Annals of Clinical Microbiology and Antimicrobials,* 4:11.

Conferences

Babaei, M.R., Sulong, A., Awang Hamat, R., Nordin, S, A., Neela, V. (2013). Extremely High Prevalence of Antiseptic Resistance *QacE* Gene among Clinical Isolates of MDR *Acinetobacter baumannii* In Malaysia. Poster presentation, International Congress of the Malaysian Society for Microbiology, 12th -15th December, Langkawi.

Babaei, M.R., Sulong, A., Awang Hamat, R., Nordin, S, A., Neela, V. (2013). Antimicrobial Susceptibility Patterns of MDR *Acinetobacter baumannii* a Major Teaching Hospital in Malaysia. Poster presentation, 9th International Symposium on Antimicrobial Agents and Resistance (ISAAR), 13th – 15th March, Kuala Lumpur.

Babaei, M.R., (2012). Participant, 7th National Infectious Diseases Seminar and Workshop (NIDSAW), University Putra Malaysia.

Babaei, M.R., (2011). Participant, Infectious diseases Seminar, 15th Nov, University Putra Malaysia.