UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF MULTI DRUG-RESISTANT PATHOGENS ISOLATED FROM CLINICAL AND ENVIRONMENTAL SAMPLES FROM NEPHROLOGY UNIT OF HOSPITAL SERDANG

SEENU SUNTHARAMURTHY

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BERILMU BERBAKTI

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By

SEENU SUNTHARAMURTHY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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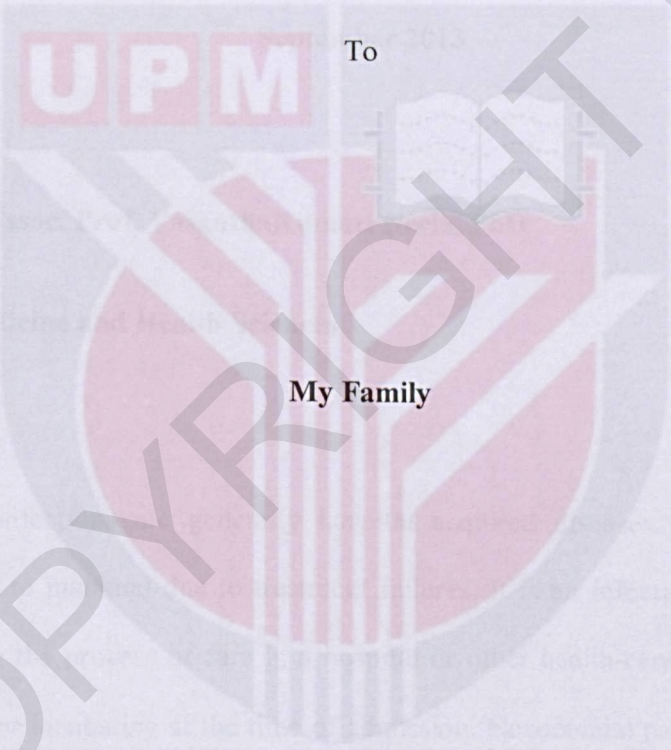
HEARTFUL DEDICATION



To

My Family

Abstract of thesis presented to the senate of University Putra Malaysia in fulfilment of the requirements of the degree of Master of Science

CHARACTERIZATION OF MULTI DRUG-RESISTANT PATHOGENS ISOLATED FROM CLINICAL AND ENVIRONMENT SAMPLES FROM NEPHROLOGY UNIT OF HOSPITAL,SERDANG

BY

SEENU SUNTHARAMURTHY

September 2013

Chairman: Assoc. Prof. VasanthaKumari Neela, PhD

Faculty: Medicine and Health Sciences

Nosocomial infections are generally hospital acquired diseases, and is posing a serious threat to mankind due to treatment failures. It is an infection occurring in a patient during the process of care in a hospital or other health-care facility that was not manifest or incubating at the time of admission. Nosocomial pathogens are often multiple drugs resistant and their emergence leads to difficulty in treatment of diseases. The common nosocomial pathogens are Methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum beta-lactamases (ESBL) *Escherichia coli* and *Klebsiella pneumoniae*, multi-drug resistant *Pseudomonas aeruginosa* and multi-drug resistant *Acinetobacter baumannii*. Nosocomial pathogens are continually evolving in resistance against antibiotics despite proper management control. The main aim of this study is to characterize multi drug resistant (MDR) pathogens isolated from

clinical and non-clinical sources isolated from the Nephrology unit in Hospital Serdang. Among the objectives were to determine phenotypic and genotypic characteristics of the common nosocomial pathogens isolated, to determine the antimicrobial susceptibility pattern and resistant genes, and finally to determine the clonality of the MDR pathogens. Nasal and hand swabs were obtained from the healthcare workers, their belonging and ward environment. All swabs were screened for the common MDR pathogens using standard procedure. In order to characterize the pathogens, all MDR clinical isolates isolated from patients admitted in the Nephrology from September 2011 to May 2012 were collected. DNA extraction was performed and antibiotic resistant genes such as *mecA, tem, shv, OXA-23, OXA-51, Int1, sul1, qacA, qacE* and *bla-IMP* coding for methicillin resistance, cephalosporinases, carbapenemases, and quaternary ammonium compounds were screened using simple PCR. Among the four sampling groups (nasal and hands of healthcare workers, their personal belonging and the ward environment), 43 isolates were *S. aureus*, 9 were *P. aeruginosa*, 23 were *K. pneumoniae*, 10 were *E. coli* and 16 were *A. baumannii*. Antimicrobial Susceptibility Testing (AST) performed on all isolates to screen for the MDR strains showed the following results: *S. aureus* showed high resistance to penicillin (51.16%), followed by cefoxitin and erythromycin (16.28%); *P. aeruginosa* showed high resistance to chloramphenicol (63.64%), followed by ceftazidime (36.36%); *A. baumannii* showed high resistance to ceftazidime and chloramphenicol (43.75% each), followed by ampicillin-sulbactam (31.25%); *E. coli* showed high resistance to ampicillin (50.0%) and co-trimoxazole (50.0%), while augmentin, aztreonam and ceftriaxone showed 40% resistance; *K. pneumoniae* showed 100% resistance to ampicillin and augmentin. All resistant isolates were screened for antibiotic resistant genes. Among the clinical isolates, one

MRSA isolate carried the *mecA* and *smr* gene. The genes *tem* and *shv* were observed in ESBL *E. coli*. One isolate showed positive signal for the *shv* gene and negative for *tem*. Two isolates showed positive signal for the *tem* gene but negative for *shv*. Although resistance was observed for *P. aeruginosa* in AST, no resistant genes were detected. Only one isolate of *A. baumannii* showed positive signal for *tem, bla-IMP, OXA-23* and *OXA-51*. All genes were confirmed by sequence analysis. PFGE was performed to trace any transmission of MDR pathogens from healthcare workers or the ward environment to the patients. All MDR pathogen isolated from clinical and non-clinical samples were fingerprinted by PFGE method (five isolates of *P. aeruginosa*, six isolates of *S. aureus*, and three isolates of ESBL *E. coli*). The dendrogram generated showed a vast diversity among the strains. No close relatedness was observed between the isolates from the HCWs, their belongings, and the environment with the clinical isolates. Further study need to be conducted including more wards from the hospital to understand the epidemiology of nosocomial pathogens in the hospital.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat kelulusan Ijazah Master Sains

PENCIRIAN PATOGEN RINTANGAN PELBAGAI DADAH YANG DIASINGKAN DARIPADA SAMPEL KLINIKAL DAN PERSEKITARAN DARI UNIT NEFROLOGI DI HOSPITAL SERDANG

OLEH

SEENU SUNTHARAMURTHY

September 2013

Pengerusi: Prof. Madya Vasanthakumari Neela, PhD

Fakulti: Perubatan dan Sains Kesihatan

Jangkitan nosokomial secara umumnya merupakan penyakit yang diperolehi dari hospital dan menimbulkan ancaman yang serius kepada umat manusia kerana kegagalan rawatan. Ia adalah infeksi yang menjangkiti pesakit yang telah dimasukkan ke hospital untuk penyakit lain. Patogen nosokomial adalah pada kebiasaannya mempunyai daya rintangan terhadap ubatan dan kemunculannya membawa kepada kesukaran dalam merawat penyakit. Patogen nosokomial biasa ialah *Methicillin-resistant Staphylococcus aureus* (MRSA), Spektrum meluas beta-lactamase (ESBL) *Escherichia coli* dan *Klebsiella pneumoniae, Pseudomonas aeruginosa* dan *Acinetobacter baumannii* rintangan pelbagai ubat. Patogen nosokomial sentiasa berevolusi dalam rintangan terhadap dadah walaupun kawalan pengurusan yang baik dijalankan. Tujuan utama kajian ini adalah untuk mengkaji ciri-ciri pathogen

rintangan pelbagai dadah yang diasingkan daripada klinikal dan persekitaran dari unit Nefrologi di Hospital Serdang. Objektif-objektif kajian ini adalah untuk mengenalpasti ciri-ciri fenotip dan genotip patogen nosokomial biasa, mengenalpasti corak kerentanan antimikrobial dan gene rintangan dan akhirnya, menentukan klonaliti patogen MDR. Swab hidung dan tangan diperolehi daripada pekerja penjagaan kesihatan, barangan milik mereka dan dari persekitaran wad. Semua swab disaringkan untuk patogen MDR biasa dengan menggunakan prosedur piawaian. Dalam usaha mengesan sumber untuk transmisi nosokomial, semua isolat klinikal MDR dari wad Nefrologi dikumpulkan dan diasingkan dari September 2011 hingga Mei 2012. Pengekstrakan DNA dilakukan dan gen rintangan antibiotik seperti *mecA, tem, shv, OXA-23, OXA-51, Int1, sul1, qacA, qacE* dan *bla-IMP* yang mengekod untuk penisillinase, cephalosporinase, carbapenemase, sebatian ammonia kuarternari disaringkan menggunakan kaedah PCR mudah. Antara empat kumpulan persampelan (hidung dan tangan pekerja, barangan milik mereka dan persekitaran wad), 43 isolat adalah *S. aureus*, 9 adalah *P. aeruginosa*, 23 adalah *K. pneumonia*, 10 adalah *E. coli* dan 16 adalah *A. baumanii*. Ujian kerentanan antimikrobial dilakukan untuk saringan spesis MDR menunjukkan keputusan yang berikut: *S. aureus* menunjukkan rintangan yang tinggi terhadap penisilin (51.16%), diikuti oleh cefoxitin dan erythromycin (16.28%); *P. aeruginosa* menunjukkan rintangan yang tinggi terhadap chloramphenicol (63,64%), diikuti oleh ceftazidime (36.36%); *A. baumannii* menunjukkan rintangan yang tinggi terhadap ceftazidime dan chloramphenicol (43.75% masing-masing), diikuti oleh ampicillin-sulbactam (31.25%); *E. coli* menunjukkan rintangan yang tinggi terhadap ampicillin (50.0%) dan cotrimoxazole (50.0%), manakala augmentin, aztreonam dan ceftriaxone menunjukkan rintangan 40%; *K. pneumoniae* menunjukkan rintangan 100% terhadap ampicillin dan

augmentin. Semua isolat rintangan telah disaringkan untuk pengesanan gen rintangan antibiotik menggunakan kaedah PCR. Antara isolat-isolat klinikal yang diperolehi, satu isolat MRSA membawa *mecA* dan gen *smr*. Gen *tem* dan *shv* diperhatikan dalam ESBL *E. coli*. Satu isolat menunjukkan isyarat positif kepada gen *shv* dan negatif untuk *tem*. Dua isolat menunjukkan isyarat positif untuk gen *tem* tetapi negatif untuk *shv*. Walaupun rintangan diperhatikan untuk *P. aeruginosa* semasa ujian kerentanan antimikrobial, tiada gen rintangan yang dikesan. Hanya satu isolat *A. baumannii* menunjukkan isyarat positif untuk *tem, bla-IMP, OXA-23* dan *OXA-51*. Semua identiti gen disahkan melalui analisis jujukan. PFGE dijalankan untuk mengesan transmisi patogen MDR daripada pekerja penjagaan kesihatan atau persekitaran wad kepada pesakit. Semua patogen MDR yang diasingkan daripada sampel klinikal dan bukan klinikal menjalani proses analisis pencapjarian menggunakan kaedah PFGE (lima isolat *P. aeruginosa*, enam isolat *S. aureus*, dan tiga isolat ESBL *E. coli*). Dendogram yang dijanakan menunjukkan kepelbagaian yang meluas diantara setiap spesies bakteria. Tiada pertalian rapat diperhatikan diantara isolat-isolat daripada pekerja penjagaan kesihatan, harta benda mereka, dan persekitaran wad dengan isolat klinikal. Kajian yang lebih mendalam perlu dijalankan termasuk liputan pelbagai wad di hospital untuk memahami epidemiologi patogen nosokomial di hospital.

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'Jai Maa Kaali'

'Om Namah Shivayah'

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I certify that a Thesis Examination Committee has met on 13 September 2013 to conduct the final examination of Seenu Suntharamurthy on his thesis entitled "Characterization of Multiple Drug-Resistant Pathogens Isolated from Clinical and Environmental Samples from the Nephrology Unit of Hospital Serdang, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Latifah binti Saiful Yazan, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Zamberi bin Sekawi, MD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd Nasir bin Mohd Desa, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Habsah Hasan, MD
Associate Professor
Universiti Sains Malaysia
Malaysia
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 20 November 2013

This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of requirement for Degree of Master of Science. The members of the Supervisory Committee were as follows:

VasanthaKumari Neela, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

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

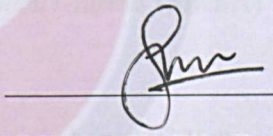
Syafinaz Amin Nordin, MD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 DEC 2013

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



SEENU SUNTHARAMURTHY

Date: 13 September 2013

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A. baumannii	*Acinetobacter baumannii*
ABC	ATP-binding cassette
AK	Amikacin
AMC	Augmentin
AMP	Ampicillin
AST	Antimicrobial Susceptibility Test
ATCC	American Type Culture Collection
ATM	Aztreonam
BLAST	Basic Local Alignment Search Tool
Bp	base pair
C	Chloramphenicol
CAPD	Continuous ambulatory peritoneal dialysis
CAZ	Ceftazidim
CEP	Cefperazone
CIP	Ciprofloxacin
CLABSI	Central line and bloodstream infection
CLSI	Clinical and Laboratory Standard Institute
CRF	Chronic renal failure
CSF	Cerebrospinal fluid
CRO	Ceftriaxone
CTX	Cefotaxime
CXM	Cefuroxime
DA	Daptomycin
E	Erythromycin

E. coli	*Escherichia coli*
EDTA	Ethylenediamine tetraacetate
ESBL	Extended Spectrum Beta Lactamase
FD	Fusidic acid
FOX	Cefoxitin
GN	Gentamicin
HAI	Hospital-acquired infection
HCW	Healthcare workers
ICU	Intensive care unit
IMP	Imipenem
K. pneumoniae	*Klebsiella pneumoniae*
K. oxytoca	*Klebsiella oxytoca*
LPS	Lipo-polysaccharide
MATE	Multidrug and toxic compound extrusion
MDR	Multiple Drug Resistant
MF	Major facilitator
MEM	Meropenem
MRSA	Methicillin resistant *Staphylococcus aureus*
NET	Netilmicin
NNIS	National nosocomial infections surveillance system
OXA	Oxacillin
P	Penicillin
P. aeruginosa	*Pseudomonas aeruginosa*
PB	Polymyxin B
PBP	Penicillin-binding protein
PDR	Pan drug resistant
PRL	Piperacillin

qac	Quatenary Ammonium Compounds
RD	Rifampin
RND	Resistance-nodulation-cell division
SAM	Ampicillin-sulbactam
S. aureus	*Staphylococcus aureus*
smr	Staphylococcal/small multidrug resistance
SCCmec	Staphylococcal cassette chromosome
shv	Sulfhydryl variable
SSI	Surgical site infection
SXT	Cotrimoxazole
TBE	Tris Borate EDTA
TE	Tris EDTA
tem	Temoneira
TZP	Piperacillin-tazobactam
VA	Vancomycin
VAP	Ventilation-associated pneumonia
x g	Centrifugal force
XDR	Extensively drug resistant

CHAPTER I

INTRODUCTION

Nosocomial infection, also known as hospital-acquired infection (HAI) is defined as an infection occurring in a patient during the process of care in a hospital or other health-care facility that was not manifest or incubating at the time of admission. This may include infections acquired in the hospital and any other setting where patients receive health care and may appear even after discharge. Nosocomial infection also includes occupational infections among facility staff (WHO, 2002). The infection sources in a hospital may be from other patients, healthcare workers, contaminated objects, or from the patient itself. Most common nosocomial infections are surgical wound infections, urinary and respiratory tract infections, and bacteremia.

Nosocomial infection which is one of the leading causes of death and increased morbidity for hospitalized patients (WHO, 2002) was often linked with antibiotic-resistant bacteria pathogens. According to National Healthcare Safety Network (Centre of Disease Control, 2008), general hospitals reported the highest number of nosocomial infections (93.9%), of which 87% were bacteria related infection. *Staphylococcus aureus* (15%), *Enterococcus* spp (12%), *Escherichia coli* (10%), and *Pseudomonas aeruginosa* (8%) were the common nosocomial pathogens. In addition to the significant morbidity and mortality burdens, nosocomial infections are associated with higher healthcare costs (Stone *et al.,* 2002; Dulworth and Pyenson, 2004; Chen *et al.,* 2005).

Risk factors for nosocomial infections include weak immunity among patients and invasive medical procedures which creates potential transmission routes for multi drug-resistant (MDR) pathogens.

Among the nosocomial pathogens, Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) (Chambers and DeLeo, 2009) and Gram-negative Extended Spectrum Beta-Lactamases (ESBL) *Escherichia coli*, MDR *Acinetobacter*, and *Pseudomonas* spp ranks top. Gram-negative bacilli resistance is mediated by β-*lactamase* enzymes that are classified into two important groups: Extended Spectrum Beta-Lactamases (ESBL) and the inducible chromosomal β-*lactamase* (Schwaber *et al.,* 2005). The strains with ESBLs are reported mainly among *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis,* and other species such as *Enterobacter* spp. and *Citrobacter freundii* (Thomson and Smith, 2000). Chromosomal β-lactamase is described in non fermenting Gram-negative bacilli such as *Enterobacter* spp., *Pseudomonas* spp., and *Acinetobacter baumannii.*

Nephrology ward in hospitals are no exceptions to nosocomial infections. Among chronic haemodialysis patients, approximately 25% of blood stream infections are caused by gram-negative bacteria (Marr *et al.,* 1997) and this percentage is increasing steadily (National Institute of Health, 2000). Treatment failure is mainly due to pathogens resistant to commonly administered drugs similar to any other wards. Patients who require hospitalization for management and renal diseases are vulnerable to nosocomial infection due to invasive devise usage, frequent haemodialysis and immune-modulators or immune-suppression therapy. The nature of

nosocomial infection and its epidemiology in patients with diabetes and end stage renal failure is not well discussed despite of high number of cases reported in Malaysia.

Common problems associated with nosocomial pathogens are its resistance towards commonly administered drugs, its mechanisms of resistance, and the main source of transmission. The source is important in epidemiological studies to enable proper management in pathogen elimination. Since nosocomial infection act as in a chain formation, the elimination of the source may actually decrease the rate of nosocomial infections in the ward. In the current study, the problem of source tracing is highlighted and the main aim is to determine the prevalence of nosocomial pathogens and to understand the source of transmission in the Nephrology ward in a recently established hospital in Malaysia.

The general objective is to characterize multiple drug resistant pathogens isolated from clinical and environmental samples in the hospital while the specific objectives are:

(1) To determine the phenotypic and genotypic characteristics of common nosocomial pathogens isolated from patients, healthcare workers (HCWs) and environment.

(2) To determine the antimicrobial susceptibility pattern of common nosocomial pathogens and the common resistant genes in the MDR pathogens.

(3) To determine the clonality of the MDR pathogens isolated from patients, healthcare workers (HCWs) and environment.

REFERENCES

Alekshun, M.N., and Levy, S.B. 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell* 128(6): 1037-1050.

Alhetar, K.Y.A., Sekawi, Z., Mariana, N.S., and Neela, V. 2011. Molecular characterization of extended-spectrum beta-lactimase (ESBL) producing extra-intestinal pathogenic *Escherichia coli*. *African Journal of Microbiology Research* 5(31): 5662-5668.

Allegranzi, B., Nejad, S.B., Combescure, C., Graafmans, W., Attar, H., Donaldson, L., and Pittet, D. 2011. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *The Lancet* 377(9761): 228-241.

Allegranzi, B., and Pittet, D. 2008. Preventing infections acquired during health-care delivery. *Lancet* 372: 1719–20.

Al-Jasser, A.M. 2006. Extended-spectrum beta-lactamases (ESBLs): a global problem. *Kuwait Medical Journal* 38(3): 171-85.

Aloush, V., Navon-Venezia, S.Y., Seigman-Igra, S., Cabili, and Carmeli, Y. 2006. Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. *Journal of Antimicrobial Agentsand Chemotherapy.* 50: 43–48.

Ambler, R.P., Coulson, A.F.W., Frere, J.M., Ghuysen, J.M., Joris, B., Forsman, M., Levesque, R.C., Tiraby, G., and Waley, S.G. 1991. A standard numbering scheme for the class A -lactamases. *Biochemical Journal* 276: 269–270.

Apisarnthanarak, A., Pinitchai, U., Thongphubeth, K., Yuekyen, C., Warren, D.K., and Fraser, V.J. 2008. A multifaceted intervention to reduce pan drug-resistant *Acinetobacter baumannii* colonization and infection in 3 intensive care units in a Thai tertiary care center: a 3-year study. *Clinical Infectious Diseases* 47: 760–767.

Arbeit, R.D. 1995. Laboratory procedures for the epidemiologic analysis of microorganism. In: Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H., eds. Manual of Clinical Microbiology. 6th ed. Washington, DC: *American Society for Microbiology* 190-208

Babini, G.S., and Livermore, D.M. 2000. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997-1998. *Journal of Antimicrobial Agents and Chemotherapy* 45: 183-189.

Ball, L.M., Bes, M.A., Theelen, B., Boekhout, T., Egeler, R.M. and Kuijper, E.J. 2004. Significance of amplified fragment length polymorphism in identification and epidemiological examination of Candida species colonization in children undergoing allogeneic stem cell transplantation. *Journal of Clinical Microbiology* 42: 1673–1679.

Banerjee, D., Satpathi, P., Pathak, T.K., Sengupta, M., and SenGupta, M. 2011. A Silent Invader–Acinetobacter: Picture of a Peripheral Medical College and Hospital of Eastern India. *Archives of Clinical Microbiology*, 2(4).

Baraniak, A., Fiett, J., Hryniewicz, W., Nordmann, P., and Gniadkowski, M. 2002. Ceftazidime-hydrolyzing CTX-M-15 extended-spectrum beta-lactamase (ESBL) in Poland. *Journal of Antimicrobial Chemotherapy* 50: 393-396.

Barlow, R.S., Desmarchelier, P.M., and Gobius, K.S. 2004. Isolation and characterization of integron-containing bacteria without antibiotic selection. *Antimicrobial Agents and Chemotherapy* 48: 838-842.

Bartual, S.G., Seifert, H., Hippler, C., Luzon, M.A.D., Wisplinghoff, H., and Rodríguez-Valera, F. 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *Journal of Clinical Microbiology* 43(9): 4382-4390.

Baumann, P., Doudoroff, M., and Stanier, R.Y. 1968. A study of the *Moraxella* group. II. Oxidase-negative species (genus *Acinetobacter*). *Journal of Bacteriology* 95: 1520–1541.

Berger-Bachi, B., and Rohrer, S. 2002. Factors influencing methicillin resistance in *staphylococci*. *Archive of Microbiology* 178(3): 165-71.

Bert, N., and Lambert-Zechovsky, N. 1996. Sinusitis in mechanically ventilated patients and its role in the pathogenesis of nosocomial pneumonia. *European Journal of Clinical Microbiology and Infectious Diseases* 15: 533–544

Blanc, D.S., Petignat, C., Janin, B., Bille, J., and Fancioli, P. 1998. Frequency and molecular diversity of *Pseudomonas aeruginosa* upon admission and during hospitalization: a prospective epidemiologic study. *Clinical Microbiology of Infection* 4: 242–247.

Bonnet, R. 2004. Growing group of extended-spectrum beta-lactamases: the *CTX-M* enzymes. *Antimicrobial Agents and Chemotherapy* 48: 1-14.

Boucher, Y.L.M., Koenig, J.E., Stokes, H.W. 2007. Integrons: mobilizable platforms that promote genetic diversity in bacteria. *Trends in Microbiology* 15: 301-309.

Bougnoux, M.E., Tavanti, A., Bouchier, C., Gow, N.A., Magnier, A., Davidson, A.D., Maiden, M.C., D'Enfert, C. and Odds, F.C. 2003. Collaborative consensus for optimized multilocus sequence typing of *Candida albicans*. *Journal of Clinical Microbiology* 41: 5265–5266.

Bradford, P.A. 2001. Extended spectrum beta-lactamases in the 21st century: characterization, epidemiology and the detection of this important resistance threat. *Clinical Microbiology Review* 14: 933-951.

Bradford, P.A, Yang, Y., Sahm, D., Grope, I., Gardovska, D., and Storch, G. 1998. CTX-M-5, a novel cefotaxime-hydrolyzing beta-lactamase from an outbreak of *Salmonella typhyimurium* in Latvia. *Journal of Antimicrobial Agents and Chemotherapy* 42: 1980-1984.

Brink, A., Feldman, C., Richards, G., Moolman, J., and Senekal, M. 2008. Emergence of extensive drug resistance (XDR) among Gram-negative bacilli in South Africa looms nearer. *South African Medical Journal* 98: 586, 8, 90.

Brooks, G.F., Butel, J.S., and Morse, S.A. 2007. Jawetz, Melnick, and Adelberg's medical microbiology. New York, NY, USA: McGraw-Hill Medical.

Burke, J.P. 2003. Infection Control — A Problem for Patient Safety. *North England Journal of Medicine* 348(7): 651-656.

Bush, K., Macalintal, C., Rasmussen, B.A., Lee, V.J., and Yang, Y. 1993. Kinetic interactions of tazobactam with beta-lactamases from all major structural classes. *Journal of Antimicrobial Agents and Chemotherapy* 37: 851-858.

Cao, V., Lambert, T., Nhu, D.Q., *et al*. 2002. Distribution of extended-spectrum beta-lactamases in clinical isolates of *Entobacteriaceae* in Vietnam. *Journal of Antimicrobial Agents and Chemotherapy* 46: 3739- 3743.

Carmeli, Y., Troillet, N., Karchmer, A.W., and Samore, M.H. 1999. Health and economic outcomes of antibiotic resistant *Pseudomonas aeruginosa*. *Archives of International Medicine* 159: 1127– 1132.

Centres for Disease Control and Prevention 1999. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1990–May 1999. *American Journal of Infection Control* 27: 520–532.

Centers for Disease Control and Prevention 2008. *S. aureus* and MRSA Surveillance Summary.http://www.cdc.gov/mrsa/library/MRSA-Surveillance Summary.html. Accessed 4 November 2010.

Chambers, H.F., and DeLeo, F.R. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Review Microbiology* 7: 629-41.

Chang, L., Chen, H., Chang, C., Lee, T.M., and Wu, W. 2004. Contribution of integrons, and SmeABC and SmeDEF efflux pumps to multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*. *Journal of Antimicrobials and Chemotherapy* 53: 518-521.

Chen, K.W., Lo, H.J., Lin, Y.H., and Li, S.Y. 2005. Comparison of four molecular typing methods to assess genetic relatedness of *Candida albicans* clinical isolates in Taiwan. *Journal of Medical Microbiology* 54(3): 249-258.

Chen, Y.C., Chang, S.C., Tai, H.M., Hsueh, P.R. and Luh, K.T. 2001. Molecular epidemiology of Candida colonizing critically ill patients in intensive care units. *Journal Formos Medical Association* 100: 791–797.

Chen, Y.Y., Chou, Y.C., and Chou, P. 2005. Impact of nosocomial infection on cost of illness and length of stay in intensive care units. *Infection Control and Hospital Epidemiology* 26(3): 281-287.

Chiou, C.S., Liao, T.L., Wang, T.H., Chang, H.L., Liao, J.C., and Li, C.C. 2004. Epidemiology and molecular characterization of *Streptococcus pyogenes* recovered from scarlet fever patients in central Taiwan from 1996 to 1999. *Journal of Clinical Microbiology* 42: 3998–4006.

Clarridge, J.E. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Review* 17: 840–862.

Clemons, K.V., Feroze, F., Holmberg, K. and Stevens, D.A. 1997.Comparative analysis of genetic variability among *Candida albicans* isolates from different geographic locales by three genotypic methods. *Journal of Clinical Microbiology* 35: 1332–1336.

Cockerill, F.R. (Ed.). (2011). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement. Clinical and Laboratory Standards Institute (CLSI).

Cohen, A.L., Calfee, D., Fridkin, S.K., *et al.* 2008. Recommendations for metrics for multidrug-resistant organisms in healthcare settings: SHEA/ HICPAC Position paper. *Infection Control and Hospital Epidemiology* 29: 901– 913.

Cosgrove, S.E., Sakoulas, G., Perencevich, E.N., Schwaber, M.J., Karchmer, A.W., and Carmeli, Y. 2003. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clinical Infectious Diseases* 36(1): 53-9.

Danel, F., Hall, L.M., Gur, D., Akalin, H.E., and Livermore, D.M. 1995(a). Transferable production of PER-1 beta-lactamase in *Pseudomonas aeruginosa*. *Journal of Antimicrobial Agents and Chemotherapy* 35: 281–294.

Danel, F., Hall, L.M.C., Gur, D., and Livermore, D.M. 1995(b). OXA-14, another extended - spectrum variant of OXA-10 (PSE-2) ß-lactamase from *Pseudomonas aeruginosa*. *Journal of Antimicrobial Agents and Chemotherapy* 39: 1881-1884.

Danel, F., Hall, L.M.C., Gur, D., and Livermore, D.M. 1998. OXA-16, a further extended-spectrum variant of OXA-10 B-lactamase, from two *Pseudomonas aeruginosa* isolates. *Journal of Antimicrobial Agents and Chemotherapy* 42: 3117-3122.

Danel, F., Hall, L.M.C., Duke, B., Gur, D., and Livermore, D.M. 1999. OXA-17, a further extended-spectrum variant of OXA-10 B-lactamase, isolated from *Pseudomonas aeruginosa*. *Journal of Antimicrobial Agents and Chemotherapy* 43: 1362-1366.

Deurenberg, R.H., and Stobberingh, E.E. 2008. The evolution of *Staphylococcus aureus*. *Infection, Genetics and Evolution* 8(6): 747-63.

Dhabaan, G.N., Hamimah, H., and Shorman, M.A. 2011. Emergence of extensive drug-resistant *Acinetobacter baumannii* in North of Jordan. *African Journal of Microbiology Research* 5(9): 1070-1075.

Diancourt, L., Passet, V., Verhoef, J., Grimont, P.A., and Brisse, S. 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *Journal of Clinical Microbiology* 43(8): 4178-4182.

Dimatatac, E.L., Alejandria, M.M., Montalban, C., Pineda, C., Ang, C., and Delino, R. 2003. Clinical outcomes and costs of care of antibiotic resistant *Pseudomonas aeruginosa* infections. *Philippine Journal of Microbiology and Infectious Diseases* 32: 159–167.

Doi, Y., Husain, S., Potoski, B.A., McCurry, K.R., and Paterson, D.L. 2009. Extensively drug-resistant *Acinetobacter baumannii*. *Emergency Infectious Diseases* 15: 980–982.

Ducel, G., *et al.* 2008. *Guide pratique pour la lutte contrel'infection hospitalière*. WHO/BAC/79.1.

Dulworth, S., and Pyenson, B. 2004. Healthcare-associated infections and length of hospital stay in the Medicare population. *American Journal of Medical Quality* 19(3): 121-127.

ECDC. European Centre for Disease Prevention and Control. Annual Epidemiological Report on Communicable Diseases in Europe 2008.http://www.ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispF orm.aspx?I D=328. 2008.

El Harrif-Heraud, Z., Arpin, C., Benliman, S., and Quentin, C. 1997. Molecular epidemiology of a nosocomial outbreak due to SHV-4 producing strain of *Citrobacter diversus*. *Journal of Clinical Microbiology* 35: 2561-2567.

Elias Costa, M.R., Carnovale, S. and Relloso, M.S. 1999. Oropharyngeal candidosis in AIDS patients: an epidemiological study using restriction analysis of *Candida albicans* total genomic DNA. *Mycoses* 42: 41–46.

Enright, M.C., and Spratt, B.G. 1999. Multilocus sequence typing. *Trends in Microbiology* 7: 82–487.

Erol, S., Altoparlak, U., Akcay, M.N., Celebi, F., and Parlak, M. 2004. Changes of microbial flora and wound colonization in burned patients. *Burns* 30: 357–361.

Falagas, M.E., Koletsi, P.K., Bliziotis, I.A. 2006. The diversity of definitions of multidrug resistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Journal of Medical Microbiology* 55: 1619–1629.

Falagas, M.E., and Karageorgopoulos, D.E. 2008. Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among gram-negative bacilli: need for international harmonization in terminology. *Clinical Infectious Disease* 46: 1121–1122.

Feizabadi, M.M., FathoIlahzadeh, B., and Taherikalani, M. 2007. Antimicrobial susceptibility patterns and distribution of bla-OXA genes among *Acinetobacter spp* isolated from patients at Tehran hospitals. *Japan Journal of Infectious Diseases* 2: 274-278.

Fluit, A.D.C., Visser, M.R., and Schmitz, F.J. 2001. Molecular detection of antimicrobial resistance. *Clinical Microbiology Review 14*: 836- 71.

Fluit, A.C., Verhoef, J., and Schmitz, F.J. 2001. Frequency of isolation and antimicrobial resistance of gram-negative and gram-positive bacteria from patients in intensive care units of 25 European university hospitals participating in the European arm of the SENTRY Antimicrobial Surveillance Program 1997–1998. *European Journal of Clinical Microbiology and Infectious Diseases* 20: 617–625.

Franco, M.R., Caiaffa-Filho, H.H., Burattini, M.N., and Rossi, F. 2010. Metallo-beta- lactamases among imipenem-resistant *Pseudomonas aeruginosa* in a Brazilian university hospital. *Clinics* 65(9): 825-829.

Gasink, L.B., Fishman, N.O., Weiner, M.G., Nachamkin, I., Bilker, W.B., and Lautenbach, E. 2006. Fluoroquinolone-resistant *Pseudomonas aeruginosa*: assessment of risk factors and clinical impact. *American Journal of Medicine* 119: 526e19–526e25.

Gautom, R.K. 1997. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. *Journal of Clinical Microbiology* 35(11): 2977-2980.

Gaynes, R.P., Edwards, J.R., Jarvis, W.R., et al. 1996. Nosocomial infections among neonates in high-risk nurseries in the United States. National Nosocomial Infections Surveillance System. *Pediatrics* 98: 357–61

Ghaznavi-Rad, E., Shamsudin, M.N., Sekawi, Z., Khoon, L.Y., Aziz, M.N., Hamat, R.A., and Neela, V. 2010. Predominance and emergence of clones of hospital-acquired methicillin-resistant*Staphylococcus aureus* in Malaysia. *Journal of Clinical Microbiology* 48(3): 867-872.

Goering, R.V. 1993. Molecular epidemiology of nosocomial infection: analysis of chromosomal restriction fragment patterns by pulsed-field gel electrophoresis. *Infection Control Hospital Epidemiology* 14: 595-600.

Goossens, H. 2003. Susceptibility of multi-drug-resistant *Pseudomonas aeruginosa* in intensive care units: results from the European MYSTIC study group. *Clinical Microbiology Infection* 9: 980–983.

Gould, I.M. 2008. The epidemiology of antibiotic resistance. *International Journal of Antimicrobial Agents* 32 (suppl1): 2–9.

Gyles, C.L., Prescott, J.F., Songer, G., and Thoen, C.O.(Eds.). 2008. Pathogenesis of bacterial infections in animals. Wiley-Blackwell.

Hall, L.M., Livermore, D.M., Gur, D., Akova, M., and Akalin, H.E. 1993. OXA-11, an extended-spectrum variant of OXA-10 (PSE-2) beta-lactamase from *Pseudomonas aeruginosa*. *Journal of Antimicrobial Agents and Chemotherapy* 37:1637-1644.

Hall, R.M., Collis, C.M., Kim, M.J., Partridge, S.R., Recchia, G.D., and Stokes, H.W. 1999. Mobile gene cassettes and integrons in evolution. *Annals of the New York Academy of Sciences* 870: 68-80

Harbarth, S., Rohner, P., Auckenthaler, R., Safran, E., Sudre, P., and Pittet, D. 1999. Impact and pattern of Gram-negative bacteremia during 6 years at a large university hospital. *Scandinavian Journal of Infectious Diseases* 31: 163—8.

Hart, C.A. 1993. *Klebsiellae* and neonates. *Journal of Hospital Infections* 23: 83–6.

Hawkey, P.M. 2008. Prevalence and clonality of extended spectrum B-lactamases in Asia. *Clinical Microbiology and Infectious Diseases* 14(1): 159-165.

Henriques, I.S., Fonseca, F., Alves, A., Saavedra, M.J., and Correia, A. 2006. Occurrence and diversity of integrons and B-lactamase genes among ampicillin-resistant isolates from estuarine waters. *Research in Microbiology* 157(10): 938-947.

Hidron, A.I., Edwards, J.R., Patel, J., Horan, T.C., Sievert, D.M., Pollock, D.A., and Fridkin, S.K. 2008. Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006– 2007. *Infection Control and Hospital Epidemiology* 29(11): 996-1011.

Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T., and Williams, S.T. 1994. International edition: Bergey's manual of determinative bacteriology.

Huang, Z.M., Mao, P.H., Chen, Y., Wu, L., and Wu, J. 2004. Study on molecular epidemiology of *SHV* type beta-lactamase encoding genes of multiple-drug-resistant *Acinetobacter baumannii*. *Zhonghua Liu Xing Bing Xue Za Zhi* 25: 425-427.

Hughes, A.J., Ariffin, N., Huat, T.L., Molok, H.A., Hashim, S., Sarijo, J., and Kamarulzaman, A. 2005. Prevalence of nosocomial infection and antibiotic use at a university medical center in Malaysia. *Infection Control and Hospital Epidemiology* 26(1): 100-104.

Huletsky, A., Knox, J.R., and Levesque, R.C. 1993. Role of ser-238 and Lys-240 in the hydrolysis of 3rd generation cephalosporins by SHV-type beta-lactamases probed by site-directed mutagenesis and 3-dimensional modeling. *Journal of Biological Chemistry* 268: 3690-3697.

Isabelle, P. 2003. An integron cassette encoding erythromycin esterase, ere(A), from *Providencia stuartii*. *Journal of Antimicrobial Agents and Chemotherapy* 51: 787-790.

Ito, T., Okuma, K., Ma, X.X., Yuzawa, H., and Hiramatsu, K. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resistance Updates* 6(1): 41-52.

Jacoby, G.A. and Munoz-Price, L.S. 2005. The new β-lactamases. *New England Journal of Medicine* 352: 380–91.

Jacoby, G.A. 1997. Extended-spectrum beta-lactamases and other enzymes providing resistance to oxyimino-beta-lactams. *Infectious Diseases Clinics of North America* 11: 875-887.

Jeong, H.Y., Lee, J.E., Choi, B.K. *et al.* 2007. Molecular epidemiology of community-associated antimicrobial-resistant *Staphylococcus aureus* in Seoul, Korea (2003): pervasiveness of multidrug-resistant SCCmec type II methicillin-resistant S. aureus. *Microbial Drug Resistant* 13: 178–185.

Johnson, J.K., Arduino, S.M., Stine, O.C., Johnson, J.A., and Harris, A.D. 2007. Multilocus sequence typing compared to pulsed-field gel electrophoresis for molecular typing of *Pseudomonas aeruginosa*. *Journal of Clinical Microbiology* 45(11): 3707-3712.

Jones, R.N. 2001. Pathogens: Trends over the past few years resistance patterns among nosocomial. *Chest* 119: 397-404.

Kallen, A.J., Hidron, A.I., Patel, J., and Srinivasan, A. 2010. Multidrug resistance among gram-negative pathogens that caused healthcare-associated infections reported to the National Healthcare Safety Network, 2006-2008. *Infection Control and Hospital Epidemiology* 31: 528–531.

Kanellopoulou, M., Stamos, G., Petinnelli, I., Savala, M., Tzimogianni, A., Legakis, N.J., Foustoukou, M., Papafragas, E. and Velegraki, A. 2001. Subtyping and antifungal susceptibilities of *Candida spp.* in the intensive care unit of a Greek general hospital. *International Journal of Antimicrobial Agents* 18: 179–183.

Karim, A., Poirel, L., Nagarajan, S., and Nordmann, P. 2001. Plasmid - mediated extended- spectrum beta-lactamase (CTX-M-3 like) from India and gene association with insertion sequence ISEcp1. *FEMS Microbiology Letters* 201: 237-241.

Karlowsky, J.A., Kelly, L.J., Thornsberry, C., Jones, M.E., and Sahm, D.F. 2002. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. *Journal of Antimicrobial Agents and Chemotherapy* 46: 2540–2545.

Kazama, H., Hamashima, H., Sasatsu, M., and Arai, T. 1998(a). Distribution of the antiseptic-resistance genes qacE and qacEΔ1 in Gram-negative bacteria. *FEMS microbiology letters* 159(2): 173-178.

Kazama, H., Hamashima, H., Sasatsu, M., & Arai, T. 1998(b). Distribution of the antiseptic-resistance gene qacEΔ1 in Gram-positive bacteria. *FEMS microbiology letters* 165(2): 295-299.

Kemp, R., Leatherbarrow, A.J., Williams, N.J., Hart, C.A., Clough, H.E., Turner, J., Wright, E.J., and French, N.P. 2005. Prevalence and genetic diversity of *Campylobacter spp.* in environmental water samples from a 100-square-kilometer predominantly dairy farming area. *Applied Environmental Microbiology* 71: 1876–1882

Kirby, W.M., Perry, D.M., and Bauer, A.W. 1959. Single-disk antibiotic-sensitivity testing of Staphylococci: An analysis of technique and results. *Archives of Internal Medicine* 104(2): 208.

Kollef, M.H., Solver, P., Murphy, D.M., and Trovillion, E. 1995. The effect of late-onset ventilator-associated pneumonia in determining patient mortality. *Chest* 108: 1655– 1662

Koreen, L., Ramaswamy, S.V., Graviss, E.A., Naidich, S., Musser, J.M., and Kreiswirth, B.N. 2004. *Spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *Journal of Clinical Microbiology* 42: 792–799.

Kshirsagar, A.V., Hogan, S.L., Mandelkehr, L., and Falk, R.J. 2000. Length of Stay and Costs for Hospitalized Hemodialysis Patients Nephrologists versus Internists. *Journal of the American Society of Nephrology* 11(8): 1526-1533.

Kücken, D., Feucht, H.H., and Kaulfers, P.M. 2000. Association of qacE and qacEΔ1 with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. *FEMS microbiology letters* 183(1): 95-98.

Labbate, M., Case, R.J., and Stokes, H.W. 2009. The integron/gene cassette system: an active player in bacterial adaptation. *Methods in Molecular Biology* 532: 103-125.

Landgren, M., Oden, H., Kuhn, I., Osterlund, A., and Kahlmeter, G. 2005. Diversity among 2481 *Escherichia coli* from women with community-acquired lower urinary tract infections in 17 countries. *Journal of Antimicrobial Agents and Chemotherapy* 55: 928 –937.

Lee, M.D., Sanchez, S., Zimmer, M., Idris, U., Berrang, M.E., and Mcdermott, P.F. 2002. Class 1 integron-associated tobramycin-gentamicin resistance in *Campylobacter jejuni* isolated from the broiler chicken house environment. *Journal of Antimicrobial Agents and Chemotherapy* 46: 3660-3664.

Levison, M.E. 2002. Plasmid-mediated extended-spectrum beta-lactamases in organisms other than *Klebsiella pneumonia* and *Escherichia coli*: a hidden reservoir of transferable resistance genes. *Current Infectious Disease Reports* 4: 181-183.

Levy, S.B. 1992. Active efflux mechanisms for antimicrobial resistance. *Journal of Antimicrobial Agents and Chemotherapy* 36: 695–703.

Levy, S.B., and Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine* 10: S122–S129.

Lim, K.T., Yasin, R., Yeo, C., Puthucheary, S., and Thong, K. 2009. Characterization of Multidrug Resistant ESBL-Producing *Escherichia coli* isolates from Hospitals in Malaysia. *Journal of Biomedicine and Biotechnology* 1-10.

Livermore, D.M. 1995. Beta-lactamases in laboratory and clinical resistance. *Clinical Microbiology Review* 8: 557-584.

Li, X.Z., and Nikaido, H. 2004. Efflux-mediated drug resistance in bacteria. *Drugs* 64: 159–204.

Li, X., Zhang, L., and Poole, K. 2002. SmeC, an outer membrane multidrug efflux protein of *Stenotrophomonas maltophilia*. *Journal of Antimicrobial Agents and Chemotherapy* 46: 333-343.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., and Monnet, D.L. 2012. Multidrug-resistant, extensively drug-resistant and pan drug- resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* 18(3): 268-281.

Marchandin, H., Carriere, C., Sirot, D., Jean-Pierre, H., and Darbas, H. 1999. TEM-24 produced by four different species of *Enterobacteriaceae*, including *Providencia rettgeri*, in a single patient. *Journal of Antimicrobial Agents and Chemotherapy* 43: 2069-2073.

Marr, K.A., Sexton, D.J., Conlon, P.J., Corey, G.R., Schwab, S.J., and Kirkland, K.B. 1997. Catheter-related bacteremia and outcome of attempted catheters salvage in patients undergoing haemodialysis. *Annual International Medicine* 137: 275–280

Martineau, F., Picard, F.J., Roy, P.H., Ouellette, M., and Bergeron, M.G. 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. *Journal of Clinical Microbiology* 36(3): 618-623.

Mazel, D. 2006. Integrons: agents of bacterial evolution. *Nature Review Microbiology* 4: 608- 620.

McDougal Linda, K., Christine D. Steward., George E. Killgore., Jasmine M. Chaitram., Sigrid K. McAllister., and Fred C. Tenover. 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *Journal of Clinical Microbiology* 41,11: 5113-5120.

Melzer, M., Eykyn, S.J., Gransden, W.R., and Chinn, S. 2003. Is methicillin resistant *Staphylococcus aureus* more virulent than methicillin-susceptible *S. aureus*? A comparative cohort study of British patients with nosocomial infection and bacteremia. *Clinical Infectious Diseases* 37(11): 1453-60.

Micek, S.T., Lloyd, A.E., Ritchie, D.J., Reichley, R.M., Fraser, V.J., and Kollef, M.H. 2005. *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. *Journal of Antimicrobial Agents and Chemotherapy* 49: 1306– 1311.

Moland, E.S., Black, J.A., Hossain, A., *et al*. 2003. Discovery of CTX-M like extended-spectrum-beta-lactamases in *Escherischia coli* isolates from five U.S. states. *Journal of Antimicrobial Agents and Chemotherapy* 47: 2382-3383.

Nandi, S., Maurer, J.J., Hofacre, C., and Summers, A.O. 2004. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. *Proceedings of the National Academy of Sciences of the United States of America* 101: 7118-7122.

Naseer, B.S., and Jayaraj, Y.M. 2010. Identification of multi-resistant *Staphylococcus aureus* in clinical specimens. *Research Journal of Medical Sciences* 4(3): 204-207.

National Institutes of Health: 2000 Annual Data Report: US Renal Data System, Bethesda, US Department of Health and Human Services, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2000

Nemoy, L.L., Kotetishvili, M., Tigno, J., Keefer-Norris, A., Harris, A.D., Perencevich, E.N., and Stine, O.C. 2005. Multilocus sequence typing versus pulsed-field gel electrophoresis for characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates. *Journal of Clinical Microbiology* 43(4): 1776-1781.

Noguchi, N., Nakaminami, H., Nishijima, S., Kurokawa, I., So, H., and Sasatsu, M. 2006. Antimicrobial agent susceptibilities and antiseptic resistance gene distribution among methicillin- resistant *Staphylococcus aureus* isolates from patients with impetigo and staphylococcal scalded skin syndrome. *Journal of Clinical Microbiology* 44(6): 2119of - 2125.

Noguchi, Norihisa, Junichi Suwa, Koji Narui, Masanori Sasatsu, Teruyo Ito, Keiichi Hiramatsu, and Jae-Hoon Song. 2005. Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes qacA/B and smr of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. *Journal of Medical Microbiology* 54,6: 557-565.

Nordmann, P., and Guibert, M. 1998. Extended spectrum ß-lactamases in *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy* 42: 128-131.

Nordmann, P., Mariotte, S., Naas, T., Labia, R., and Nicolas, M.H. 1993. Biochemical properties of a carbapenem-hydrolyzing B-lactamase for *Enterobacter cloacae* and cloning of the gene into *Escherichia coli*. *Journal of Antimicrobial Agents and Chemotherapy* 37: 939–946.

O'Fallon, E., Gautam, S., and D'Agata, E.M.C. 2009. Colonization with multi drug resistant gram-negative bacteria: prolonged duration and frequent co-colonization. *Clinical Infectious Disease* 48: 1375–1381.

Ohara, T., and Itoh, K. 2003. Significance of *Pseudomonas aeruginosa* colonization of the gastrointestinal tract. *International Medicine* 42: 1072–1076.

Pal Balikaran Ramprasad, Marissa Rodrigues, and Suprama Datta. 2010. Role of pseudomonas in nosocomial infections and Biological characterization of local strains. *Journal of Bioscience and Technology* 1,4: 170-179.

Park, Y.K., Peck, K.R., Cheong, H.S., Chung, D.R., Song, J.H., and Ko, K.S. 2009. Extreme drug resistance in *Acinetobacter baumannii* infections in intensive care units, South Korea. *Emergency Infectious Disease* 15: 1325–1327.

Paterson, D.L., and Bonomo, R.A. 2005. Extended-spectrum β-lactamases: a clinical update. *Clinical Microbiology Review* 18: 657–86.

Pathmanathan, S.G., Samat, N.A., and Mohamed, R. 2009. Antimicrobial susceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian Hospital. *The Malaysian Journal of Medical Sciences* 16(2): 27.

Paulsen, I.T., Brown, M.B. and Skurray, R.A. 1996. Protondependent multidrug efflux systems. *Microbiology Review* 60: 575-608.

Paulsen, I.T., Littlejohn, T.G., Raîdstroëm, P., Sundstroëm, L., Skoë ld, O., Swedberg, G. and Skurray, R.A. 1993. The 3P conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. *Journal of Antimicrobial Agents and Chemotherapy* 37: 761-768.

Perilli, M., Segatore, B., Massis, M.R.D., *et al.* 2000. TEM-72, a new extended-spectrum ß- lactamase detected in *Proteus mirabilis* and *Morganella morganii* in Italy. *Journal of Antimicrobial Agents and Chemotherapy* 44: 2537-2539.

Philippon, L.N., Nass, T., Bouthor, A.T., Barakett, V., and Nordmann, P. 1997. OXA-18, a class D clavulanic acid-inhibited extended- spectrum ß-lactamase from *Pseudomonas aeruginosa*. *Journal of Antimicrobial Agents and Chemotherapy* 41: 2188- 2195.

Pillar, C.M., Draghi, D.C., Sheehan, D.J., and Sahm, D.F. 2008. Prevalence of multidrug- resistant, methicillin-resistant *Staphylococcus aureus* in the United States: findings of the stratified analysis of the 2004 to 2005 LEADER Surveillance Programs. *Diagnostic Microbiology and Infectious Disease* 60: 221–224.

Pitt, T.L. 1998. *Pseudomonas*, *Burkholderia*, and related genera, p.1109–1138. *In* B. I. Duerden (ed.), Microbiology and microbial infections, vol. 2. Oxford University Press Inc., New York, NY.

Pittet, D., Allegranzi, B., Sax, H., Bertinato, L., Concia, E., Cookson, B. *et al.* 2005. Considerations for a WHO European strategy on health-care-associated infection, surveillance, and control. *Lancet Infectious Disease* 5: 242–50.

Podschun, R., and Ullman, U. 1998. *Klebsiella spp.* as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. *Clinical Microbiology Reviews* 11(4): 589–603.

Poikonen, E., Vuopio-Varkila, J., Kaukoranta-Tolvanen, S.S., Sivonen,A., Siren, E. and Ruutu, P. 2001. Epidemiological typing of *Candida albicans* from bloodstream infections by restriction enzyme analysis. *Scandinavian Journal of Infectious Disease* 33: 140–144.

Poirel, L., Gniadkowski, M., and Nordmann, P. 2002. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum beta-lactamase CTX-M-15 and of its structurally related beta-lactamase CTX-M-3. *Journal of Antimicrobial Agents and Chemotherapy* 50: 1031-1034

Poirel, L., Lebessi, E., Castro, M., Fevre, C., Foustokou, M., and Nordmann, P. 2004. Nosocomial outbreak of extended-spectrum beta-lactamase SHV-5-producing isolates of *Pseudomonas aeruginosa* in Athens, Greece. *Journal of Antimicrobial Agents and Chemotherapy* 48: 2277-2279.

Pollack, M. 1995. *Pseudomonas aeruginosa*, p. 1820–2003. *In* G. L. Mandell, R. Dolan, and J. E. Bennett (ed.), Principles and practices of infectious diseases. Churchill Livingstone, New York, NY.

Prinarakis, E.E., Miriagou, V., Tzelepi, E., Gazouli, M., and Tzouvelekis, L.S. 1997. Emergence of an inhibitor-resistant ß-lactamase (SHV-10) derived from an SHV-5 variant. *Journal of Antimicrobial Agents Chemotherapy* 41: 838-840.

Quale, J.M., Landman, D., Bradford, P.A., *et al.* 2002. Molecular epidemiology of a citywide outbreak of extended-spectrum B-lactamase-producing *Klebsiella pneumoniae* infection. *Clinical Infectious Disease* 35: 834–41.

Queenan, A.M., and Bush, K. 2007. Carbapenemases: the versatile ß-lactamases. *Clinical microbiology reviews* 20(3): 440-458.

Radice, M., Power, P., DiConza, J., and Gutkind, G. 2002. Early dissemination of CTX-M- derived enzymes in South America. *Journal of Antimicrobial Agents and Chemotherapy* 46: 602-604.

Recchia, G.D., and Hall, R.M. 1997. Origins of the mobile gene cassettes found in integrons. *Trends in Microbiology* 5: 389-394.

Redkar, R.J., Dube, M.P., McCleskey, F.K., Rinaldi, M.G. and Del Vecchio, V.G. 1996. DNA fingerprinting of *Candida rugosa* via repetitive sequence-based PCR. *Journal of Clinical Microbiology* 34: 1677–1681.

Riccio, M.L., Franceschini, N., Boschi, L., Caravelli, B., Cornaglia, G., Fontana, R., *et al.* 2000. Characterization of the metallo-α-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla (IMP) allelic variants carried by gene cassettes of different phylogeny. *Journal of Antimicrobial Agents and Chemotherapy* 44: 1229-35.

Riederer, K., Fozo, P. and Khatib, R. 1998. Typing of *Candida albicans* and *Candida parapsilosis*: species-related limitations of electrophoretic karyotyping and restriction endonuclease analysis of genomic DNA. *Mycoses* 41: 397–402.

Rouch, D.A., Cram, D.S., Berardino, D., Littlejohn, T.G., and Skurray, R.A. 1990. Efflux-mediated antiseptic resistance gene qacA from *Staphylococcus aureus*: common ancestry with tetracycline-and sugar-transport proteins. *Molecular microbiology* 4(12): 2051-2062.

Rowe-Magnus, D.A., and Mazel, D. 2001. Integrons: natural tools for bacterial genome evolution. *Current Opinion in Microbiology* 4: 565-569.

Ruiz, L., Domínguez, M.A., Ruiz, N., and Viñas, M. 2004. Relationship between clinical and environmental isolates of *Pseudomonas aeruginosa* in a hospital setting. *Archives of Medical Research* 35: 251-257.

Rupp, M.E., and Fey, P.D. 2003. Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. *Drugs* 63: 353–365.

Salah, B.D., Makni, S., and Redjeb, B.S. 2002. Epidemiology of Gram-negative bacterial septicemias: data from Tunisian hospital (1996—1998). *Tunisian Medicine* 80: 245—8.

Salamon, H., Segal, M.R., Ponce de Leon, A. and Small, P.M. 1998. Accommodating error analysis in comparison and clustering of molecular fingerprints. *Emergency Infectious Disease* 4: 159–168.

Samaranayake, Y.H., Samaranayake, L.P., Dassanayake, R.S., Yau, J.Y., Tsang, W.K., Cheung, B.P. and Yeung, K.W. 2003. Genotypic shuffling of sequential clones of *Candida albicans* in HIV-infected individuals with and without symptomatic oral candidiasis. *Journal of Medical Microbiology* 52: 349–359.

Savli, H., Karadenizli, A., Kolayli, F., Gundes, S., Ozbek, U., and Vahaboglu, H. 2003. Expression stability of six housekeeping genes: a proposal for resistance gene quantification studies of Pseudomonas aeruginosa by real-time quantitative RT-PCR. *Journal of Medical Microbiology* 52(5): 403-408.

Schwaber, M.J., Navon-Venezia, S., Schwartz, D., and Carmeli, Y. 2005. High levels of antimicrobial coresistance among extended spectrum-α-lactamase-producing Enterobacteriaceae. *Journal of Antimicrobial Agents and Chemotherapy* 49: 2137-9.

Seifert, H., Strate, A., and Pulverer, G. 1995. Nosocomial bacteremia due to *Acinetobacter baumannii*: clinical features, epidemiology, and predictors of mortality. *Medicine* 74: 340–349

Singh, A., Goering, R.V., Simjee, S., Foley, S.L., and Zervos, M.J. 2006. Application of molecular techniques to the study of hospital infection. *Clinical microbiology reviews* 19(3): 512-530.

Soll, D.R. 2000. The ins and outs of DNA fingerprinting the infectious fungi. *Clinical MicrobiologyReview* 13: 332–370.

Stone, P.W., Larson, E., and Kawar, L.N. 2002. A systematic audit of economic evidence linking nosocomial infections and infection control interventions: 1990-2000. *American Journal of Infection Control* 30(3): 145-152.

Talaro, K.P., and Talaro, A. 2002. Foundations in microbiology. Blacklick.

Taylor, B.N., Harrer, T., Pscheidl, E., Schweizer, A., Rollinghoff, M. And Schroppel, K. 2003. Surveillance of nosocomial transmission of *Candida albicans* in an intensive care unit by DNA fingerprinting. *Journal of Hospital Infections* 55: 283–289.

Tauch, A., Gotker, S., Puhler, A., Kalinowski, J., and Thierbach, G. 2002. The 27.8-kb R- plasmid pTET3 from *Corynebacterium glutamicum* encodes the aminoglycoside adenyltransferase gene cassette aadA9 and the regulated tetracycline efflux system Tet 33 flanked by active copies of the widespread insertion sequence IS6100. *Plasmid* 48: 117-129.

Tenover, F.C. 2006. Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine* 119(6): S3-S10.

Tenover, F.C., Arbeit, R.D., Goering, R.V., *et al.* 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology* 33: 2233-2239

Thomson, K.S. and Smith, M.E. 2000. The new α-lactamases of Gram negative bacteria at the dawn of the new millennium. *Microbiology Infection* 2: 1225-35.

Thuong, M., Arvaniti, K., Ruimy, R., de la Salmoniere, P., Scanvic-Hameg, A., Lucet, J.C., and Regnier, B. 2003. Epidemiology of *Pseudomonas aeruginosa* and risk factors for carriage acquisition in an intensive care unit. *Journal of Hospital Infection* 53: 274–282.

Tseng, Y.C., Wang, J.T., Wu, F.L., Chen, Y.C., Chie, W.C., and Chang, S.C. 2007. Prognosis of adult patients with bacteremia caused by extensively resistant *Acinetobacter baumannii*. *Diagnostic Microbiology and Infectious Disease* 59: 181–190.

Tzouvelekis, L.S., Tzelepi, E., Tassios, P.T., and Legakis, N.J. 2000. CTX–M-type-beta-lactamases: an emerging group of extended spectrum enzymes. *International Journal of Antimicrobial Agents* 14: 137- 142.

Valles, J., Mariscal, D., Cortes, P., Coll, P., Villagra, A., Diaz, E., Artigas, A., and Rello, J. 2004. Patterns of colonization by *Pseudomonas aeruginosa* in intubated patients: a 3-year prospective study of 1607 isolates using pulsed field gel electrophoresis with implications for prevention of ventilator associated pneumonia. *Intensive Care Medicine* 30: 1768–1775.

van Rensburg, B.W.J., van Staden, A.M., Rossouw, G.J., and Joubert, G. 2010. The profile of adult nephrology patients admitted to the Renal Unit of the Universitas Tertiary Hospital in Bloemfontein, South Africa from 1997 to 2006. *Nephrology Dialysis Transplantation* 25(3): 820-824.

Vincent, J.L., Bihari, D.J., Suter, P.M., *et al.* 1995. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. *Journal of American Medical Association* 274: 639–644.

Vincent, J.L., Rello, J., Marshall, J., Silva, E., Anzueto, A., Martin, C.D. *et al.* 2009. EPIC II Group of Investigators. International study of the prevalence and outcomes of infection in intensive care units. *Journal of the American Medical Association* 302: 2323–9.

Walsh, T.R. 2005. The emergence and implications of metallo-B-lactamases in Gram-negative bacteria. *Clinical Microbiology Infections* 11:2–9.

Walsh, T.R., Toleman M.A., Poirel L., and Nordmann P. 2005. Metallo-B-lactamases: the quiet before the storm? *Clinical Microbiology Review* 18: 306–325

Wang, H., Kelkar, S., Wu, W., Chen, M., and Quinn, J.I. 2003. Clinical isolates of *Enterobacteriaceae* producing extended-spectrum beta-lactamases: prevalence of CTX-M-3 at a hospital in China. *Journal of Antimicrobial Agents and Chemotherapy* 47: 790- 793.

World Health Organization; 2002. Prevention of hospital-acquired infections: a practical guide.

World Health Organization; 2009. Geneva: WHO guidelines on hand hygiene in health care.

World Health Organization; 2010. Report on the burden of health care-associated infection worldwide.

Woodford, N., Zhang, J., Kaufmann, M.E., Yarde, S., Tomas, M.D.M., Faris, C., Vardhan, M.S., Dawson, S., Cotterill, S.L., and Livermore, D.M. 2008. Detection of *Pseudomonas aeruginosa* isolates producing VEB-type extended-spectrum beta- lactamases in the United Kingdom. *Journal of Antimicrobial Agents and Chemotherapy* 62: 1265–1268.

Xu, Z., Li, L., Shi, L., and Shirtliff, M. 2011c. Class 1 integron in Staphylococci. *Molecular Biology Reports* 38: 5261-5279.

Xu, Z., Li, L., Shirtliff, M.E., Peters, B.M., Peng, Y., Alam, M.J., Yamasaki, S., and Shi, L. 2010. First report of class 2 integron in clinical *Enterococcus faecalis* and class 1 integron in *Enterococcus faecium* in South China. *Diagnostic Microbiology of Infectious Disease* 68: 315-317.

Yamasaki, Y., Komatsu, M., Yamashita, T., *et al*. 2003. Production of CTX-M-3 extended-spectrum beta-lactamases and IMP-1 metallo-beta-lactamase by five Gram-negative bacilli; survey of clinical isolates from seven laboratories collected in 1998 and 2000 in the Kinki region of Japan. *Journal of Antimicrobial Agents and Chemotherapy* 51: 631-638.

Zhanel, G.G., Dueck, M., Hoban, D.J., Vercaigne, L.M., Embil, J.M., Gin, A.S., and Karlowsky, J.A. 2001. Review of macrolides and ketolides. *Drugs* 61(4): 443-49.