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Original Article

Bacterial Spectrum and Susceptibility patterns of Pathogens in ICU and IMCU of a Secondary Care Hospital in Kingdom of Saudi Arabia

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Abstract

Objective: To evaluate the microbiological spectrum and susceptibility pattern of pathogens in intensive care unit (ICU) and intermediate care unit (IMCU) in a single medical center from June 2011 to May 2012.

Study Design: Prospective descriptive study.

Place and duration of study: The study was carried out at the Department of Microbiology, King Abdullah Hospital Bisha, Kingdom of Saudi Arabia over a period of 12 months from June 2011 to May 2012.

Materials and Methods: Antibiotic in vitro susceptibility data of predominant ICU and IMCU isolates during 2011–12 were analyzed using WHONET program.

Results: 335 Clinical isolates were analyzed. The frequencies of Gram-positive and Gram negative bacteria were 15 % and 85% respectively. *Acinetobacter spp*, *Klebsiella species* and *Pseudomonas species* were the most common Gram negative isolates, while *Staph. aureus* and *Coagulase-negative staphylococci* (CoNS) were the two leading Gram positive isolates. 81 % *Acinetobacter spp* were found Multidrug- Resistant. Three *Acinetobacter spp* were found pan resistant. Extended-spectrum beta-lactamase (ESBL) producing *Klebsiella Pneumoniae* accounted for 57 % of all *Klebsiella species* isolates. 29% *Pseudomonas aeruginosa* were found resistant to Imipenem.

Conclusion: The high incidence of reduced antibiotic susceptibility among Gram negative bacteria in ICUs suggests that more effective strategies are needed to control the selection and spread of resistant organisms.

Key words: Drug resistance, Intensive care units, Susceptibility Patterns

Introduction

Antimicrobial resistance has emerged as an important factor in predicting outcomes and overall resource use after infections in intensive care units. Globally ICUs are encountering emergence and spread of antibiotic-resistant pathogens and for some pathogens there are few therapeutic options available.[1]

Previous epidemiological studies have focused primarily on 2 common Gram positive antimicrobial resistant organisms; Methicillin-resistant *Staph aureus* (MRSA) and Vancomycin-resistant *Enterococcus* (VRE).[2]

Spellberg *et al* showed that multi drug resistance (MDR) among Gram-negative bacteria is becoming even a greater problem in health care facilities[3].

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For some pathogens there are few therapeutic options available, e.g., extended spectrum β-lactamase (ESBL)-producing gram-negative bacteria and MDR-*Acinetobacter*. Awareness of these problems has been underscored with data from a number of surveillance studies aimed at improving the use of empiric therapy. In the United States there have been several national programs, which have focused on both the etiology of infections and resistance patterns of nosocomial or ICU infections including the National Nosocomial Infections Surveillance (NNIS) [4]now known as National Healthcare Safety Network (NHSN).

More than 20% of patients admitted to European intensive care units (ICUs) develop an ICU acquired infection. A high prevalence of decreased antibiotic susceptibility among gram-negative bacilli has been reported from ICU patients in France, Belgium, and Germany, during 1990 and 1991, the United States between 1990 and the 1993, and Belgium and Sweden during 1994 and 1995.[5]

The emergence of MDR bacteria is an increasing problematic cause of health care associated infections in ICUs, not only due to increased morbidity and mortality, but also due to increased treatment costs as result of frequent empirical failure and lengthy hospital stay.[6]

Key factors in the management and prevention of MDR bacteria include rational use of antibiotics, hand hygiene, single-use items for individual patients, isolation of patients infected with resistant isolates, environmental cleaning, surveillance, active patient and resource management and education. [7, 8]

This study using WHONET program reports the antimicrobial resistance profiles of bacterial isolates from ICU and IMCU patients during the period 2011–2012.

These surveillance programs help to maintain current knowledge of local susceptibilities and relevant treatment options.

Material and Methods

The study was carried out at the Department of Microbiology, King Abdullah Hospital, Bisha over a period of 12 months from June 2011 to May 2012.

King Abdullah Hospital, Bisha, is a 400-bed referral center in Bisha region in Kingdom of Saudi Arabia. The capacity of ICU is 13 beds and that of IMCU is 7 beds. Severely ill medical and surgical patients, except for neonates are candidates for admission. The ICU has an average annual admission rate of 40 patients and an average monthly occupancy rate of 85%. The IMCU has an average annual admission rate of 14 patients and an average monthly bed occupancy rate of 87%. Patients with road traffic accidents, sepsis, respiratory tract infections, and those undergoing surgery for complicated diseases comprise the usual patient population. The patient and nurse ratio in these units is 1:1.

Cultures were taken from respiratory specimens, blood, urine, wound and CSF depending upon identifiable focus of infection. Initially strains were identified based on the morphological behavior of the isolates on various differential media. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 mm at 15 lb pressure. The respiratory specimens and CSF were inoculated onto 5% sheep blood agar, MacConkey agar, and Chocolate agar. Wound swabs were inoculated onto blood agar and MacConkey agar. Urine specimens were inoculated onto Cystiene Lactose Electrolyte Deficient (CLED) media with a calibrated loop. Blood agar, MacConkey, and CLED plates were incubated aerobically at 37°C for 18 to 24 hours. Chocolate agar plates were incubated at 37°C in 5% CO_2 for 18 to 24 hours. For blood culture 5-10 ml of blood for adult and 1-5 ml for children and was collected. Blood cultures were processed using the BACTEC 9240 blood culture system (Becton Dickinson, Maryland, USA). If growth is displaced as positive, then it is sub-cultured on appropriate media. Organism's identification and antibiotic susceptibility testing were done by using BD Phoenix Automated Microbiology system (Becton Dickinson, Maryland, USA). The ID portion of the Phoenix panel utilizes a series of conventional, chromogenic and fluorogenic biochemical tests to determine the identification of the organism. The Phoenix AST method is a broth based microdilution test. The Phoenix system utilizes a redox indicator for the detection of organism growth in presence of an antimicrobial agent Repeat isolates were not included in this analysis. Clinical Laboratory Standards (CLSI) interpretive criteria were used for susceptibility results. Susceptibility testing was performed using the modified Kirby- Bauer disk diffusion method by using Muller Hinton Agar for antibiotics, which were not on the Phoenix panels (colistin, and tigecycline). The results were expressed as susceptible/resistant according to Clinical Laboratory Standards (CLSI) interpretive criteria. Presence of Extended-spectrum beta-lactamase (ESBL) was suggested by resistance to a third generation cephalosporins (cefotaxime, ceftriaxone or ceftezidime) in Phoenix Automated system. Quality control was performed by using reference strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 to confirm consistency of materials, methods, and results.

WHONET Version 5.6 was used for compilation and calculation of data.

Definition of Resistance: MDR for Gram-negative organisms was defined as resistance to three or more classes of antimicrobial agents, while pan-drug resistant strains are those which showed resistance to all classes.[9]The MDR strains of *Mycobacterium tuberculosis* were not addressed in this study.

Results

In vitro susceptibility data from 335 isolates from ICU and IMCU over the period one year were assimilated. The most frequent species isolated from infections in ICU and IMCU was *Acinetobacter*. The most common organisms isolated are shown in **Table 1.** The frequencies of Gram-positive and Gram negative bacteria were 15 %(n=52 and 85% (n=273, respectively. *Acinetobacter spp, Klebsiella species* and *Pseudomonas aeruginosa* were the most common isolates among Gram negative organisms, while *Staph. aureus* and *Coagulase-*

negative staphylococci (CoNS) were the two leading Gram positive isolates.

Table-1: Distribution of organisms in ICU and IMCU

Name of organism	Number
Staph. aureus	31 (9.2%)
Methicillin-resistant *Staph aureus* (MRSA)	11 (3.2% among all isolates and 35% among all Staph isolates)
Coagulase Negative Staphylococci(CoNS)	15 (4.4%)
Methicillin-resistant *Coagulase Negative Staphylococci*	14(4.1% among all isolates and 93 % among all *Coagulase Negative Staphylococci* isolates)
Enterococcus species	2 (0.6%)
Other gram positive	3 (0.9%)
Acinetobacter spp	79 (24%)
MDR Acinetobacter	64/79 (81 % among all *Acinetobacter spp*)
Serratia species	5 (1.5%)
Pseudomonas aeuroginosa	67 (20%)
Stenotrophomonas maltophilia	3 (0.9%)
Morganella morgani	5 (1.5%)
E.coli	21 (6.2%)
Klebsiella species	74 (22%)
Enterobacter species	11 (3.9%)
Other gram negative	1 (0.3%)
Candida non-albican	2 (0.6%)
Candida albican	5 (1.5%)
TOTAL	335

Table 2&3 shows the percentage of antibiotics resistance in Gram-positive and Gram-negative isolates resistant to the antibiotics tested. 35% of the isolated *S aureus* and 93% of the *Coagulase-negative staphylococci* (CoNS) were methicillin resistant. However, both methicillin resistant *Staph. aureus* and *Coagulase-negative staphylococci* were showed no resistance to vancomycin. For *Enterococcus spp*, there were no VRE isolate found. The proportions of *Acinetobacter spp*, *Klebsiella species* and *Pseudomonas aeruginosa* to all isolates were 24 % (n=79), 22 %(n=74) and 20% (n=67) respectively. The highest resistance rate for *Acinetobacter spp* were: Ampicillin and Cefuroxime (100%); followed by Gentamicin(90%), Amikacin(83 %), Ceftriaxone(88 %), Ciprofloxacin(79 %), Imipenem(79 %), and Ceftazidine(86 %). 81 %(n=64) *Acinetobacter species* were found Multidrug- Resistant. Three (3.8%) *Acinetobacter species* were found pan resistant (resistant to all antibiotics including colistin). Because of limited supply of colistin disk, we used it only for the *Acinetobacter* isolates. Extended-spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* accounted for 57 %(n=42) of *Klebsiella species*. For *Pseudomonas aeuroginosa,* the resistance rates for imipenem, Ciprofloxacin, and Ceftazidine were found 29%, 35%, and 46% respectively.

Candida was the most common fungal isolate (2%, n=7) in the ICU and IMCU.

Sputum was the most common source of these isolates (54%), followed by blood (18%) urine (8%) wound swab (7%) and CSF (5%). *Acinetobacter spp*, *Klebsiella species* and *Pseudomonas aeuroginosa* were the 3 most common isolates from sputum and urine. *Coagulase Negative Staphylococci* was the most frequent isolate from blood culture.

Table-2: Resistance pattern of common used antibiotics against commonly isolated Gram positive pathogens

Name of organism	Antibiotics	Resistance
Staph. aureus	Erythromycin	40 %
	Methicillin	35 %
	Ciprofloxacin	17%
	Gentamicin	36%
	Tetracycline	42%
	Clindamycin	30 %
	Vancomycin	0 %
Coagulase Negative Staphylococci	Erythromycin	100 %
	Methicillin	93 %
	Ciprofloxacin	56%
	Gentamicin	65%
	Tetracycline	17%
	Clindamycin	73 %
	Vancomycin	0 %

Table-3: Resistance pattern of common used antibiotics against commonly isolated Gram negative pathogens

Name of organism	Antibiotics	Resistance
Acinetobacter spp	Ampicillin	100%

	Amikacin	83 %
	Gentamicin	90%
	Cefuroxime	100%
	Ceftriaxone	88 %
	Imipenem	79 %
	Ciprofloxacin	79 %
	Ceftazidine	86 %
	Colistin	3.8 %
Pseudomonas aeuroginosa	Amikacin	29 %
	Gentamicin	36%
	Imipenem	29 %
	Ciprofloxacin	35 %
	Ceftazidine	46 %
Klebsiella pneumoniae	Amoxicillin/clavulanic acid	93 %
	Amikacin	29 %
	Gentamicin	55%
	Cefuroxime	80 %
	Ceftriaxone	70 %
	Imipenem	41 %
	Ciprofloxacin	60 %
	Ceftazidine	69 %
Klebsiella species	Amoxicillin/clavulanic acid	90 %
	Amikacin	0 %
	Gentamicin	25%
	Cefuroxime	63%
	Ceftriaxone	50 %
	Imipenem	15 %
	Ciprofloxacin	40 %
	Ceftazidine	69 %
Escherichia coli	Ampicillin	92 %
	Amikacin	10 %
	Gentamicin	18%
	Cefuroxime	80 %
	Ceftriaxone	60 %
	Imipenem	0 %
	Ciprofloxacin	45 %
	Ceftazidine	52%
Enterobacter species	Ampicillin	100 %
	Amikacin	10 %
	Gentamicin	17%
	Cefuroxime	75%
	Ceftriaxone	62%
	Imipenem	0 %
	Ciprofloxacin	32 %
	Ceftazidine	42%

Discussion

Most isolates recovered from the respiratory specimens (54 %) followed by the blood specimens (18%). These findings corroborated the results reported in a study from Saudi Arabia.[2] The most common isolates observed in this study were *Acinetobacter spp, Klebsiella spp, P.aeruginosa, Staph. Aureus,* and *Coagulase-negative staphylococci.* This observation is agreed with finding of Nermin K Saeed *et al.*[2]The incidence of Gram-positive and Gram-negative bacilli in our study was 15% and 85% respectively. According to Chuon-Yi Lee *et al* the incidence of Gram positive and Gram negative was reported 30% to 47% and 40% to 48% respectively.[10]

The percentage of *S aureus* and *Coagulase-negative staphylococci* (CoNS) are 9.2% and 4.4% respectively as compared to NHSN data 2006-2007 which is 14.5% for *Staphylococcus aureus* and 15.3% for *Coagulase-negative staphylococci* (CoNS).[11] In the present study, 35% of the isolated *S aureus* and 93% of the *Coagulase-negative staphylococci* (CoNS) were methicillin resistant. This MRSA rate was similar to that reported by Mark E Jones *et al*. during a surveillance study from a French ICU[1]. The contributions of methicillin-resistant S aureus (MRSA) and *Coagulase-negative staphylococci* to hospital acquired infection were demonstrated previously.[12, 13] In Europe, surveillance data shows a marked variability among the various states, with MRSA ranging from less than 1% to more than 50%. The highest prevalence was seen in Portugal (49%), Greece (40%) and Italy (37%), where as the lowest prevalence was observed in Norway (<1%) Sweden (1%) and Holland (1%).[14] The prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) was reported more than 50% of all *Staphylococcus aureus* isolates obtained in ICUs.[15] Rupp ME *et al* also reported the resistance to methicillin in *Coagulase-negative staphylococci* (CoNS) rates of 80%.[16]*Coagulase-negative staphylococci*, once considered culture contaminants from clinical specimens, currently represent the leading cause of foreign body-associated infections.[16] In our study, all Gram positive isolates were found sensitive to vancomycin contrary to 15.5 % resistant reported by Hossam M *et al*[17] and comparable to Nermin K Saeed *et al.*[2]

The incidence of Gram negative bacteria in our study is 85% as compare to 40% to 48% reported by Chuon-Yi Lee *at al*.**[10]** Among Gram-negative pathogens; MDR *Acinetobacter*, Imepenem resistant *P. aeruginosa* and ESBL-producing *K. Pneumonia* are of great concern.

Acinetobacter spp. has recently advanced to one of the most common pathogens isolated from ICUs.

In our study *Acinetobacter spp* accounted 24% of all isolates and out of these 81% found to be MDR and 3.8% were found pandrug- resistant. These findings are consistent with the study performed by Seifert *et al*.[18]However, this rate is higher than those of previous reports of Horan TC *at al*, which was 58%.[19] Although not the most virulent gram-negative pathogen, *Acinetobacter* is an increasingly infectious threat,

especially for patients receiving broadspectrum antimicrobial therapy and requiring life support.[5] A Spanish study[20] has shown that *Acinetobacter* isolates, usually acquired in the ICU, are multidrug resistant and may cause severe infections associated with a high mortality rate. It is an important source of nosocomial septicemia, pneumonia, and urinary tract infections [21]. Reports of multidrug-resistant isolates have increased during the last decade, probably as a result of the extensive use of broad-spectrum antibiotics. [22] In many cases, these multidrug-resistant isolates are resistant to expanded-spectrum cephalosporins and carbapenems.[19, 22, 23]

The emerging pandrug-resistant *Acinetobacter spp* is an eye opener for healthcare providers. These resistance patterns reflect our first concern, which is the end of our current pharmacopoeia. Critically, it warns us that antibiotic resistance can become a global problem that requires bold and decisive global action. It is essential that such recommendations are no longer ignored but fully implemented in a transparent and accountable manner. Because of emergence of multidrug-resistance and pandrug-resistance associated with *Acinetobacter spp*, the role of preventing spread of this pathogen to other patients is paramount. The recently released Centers for Disease Control and Prevention (CDC) infection control recommendations indicate that hospitals with increased rates of multidrug-resistant *Acinetobacter* should take more aggressive infection control measures to control and prevent further nosocomial transmission.[24] Implementation of aggressive infection control measures can control outbreaks of *Acinetobacter* infections in healthcare settings.[25, 26, 27]

ESBL-Producing *Klebsiella pneumoniae* has been increasing incremently since 2005.The icidence of 57% in our study is higher than previous reports from Taiwan.[28] We did not find ESBL-Production in other GNRs, the reason may be the small sample size. ESBL-producing isolates should be reported as resistant to all penicillins, cephalosporins, and aztreonam .[29]Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms. Consequently, there is intensive use of carbapenems as first choice antibiotics for these organisms, resulting in the emergence of carbapenem-resistant isolates and leaving at best only two therapeutic options: colistin and tygecycline.[30]

In our study the resistance rates of *Pseudomonas aeruginosa* to imipenem, Ciprofloxacin, and Ceftazidine were found 29%, 35%, 46% respectively. These findings are consistent with the data reported by Nermin K Saeed *et al*.[2] Rate of *Pseudomonas aeruginosa* resistant to carbapenem is also comparable to NNIS data [31] and lower than those of previous reports in Taiwan at 66%.[32] Possible reasons for high resistance rate for carbapanem and other antimicrobial agents in *P.aeruginosa* include more critical ill patients admitted to ICU; more patients being referred from local hospitals; and the spread of resistant strains from adult wards. It has been reported that MDR strains of *P.aeruginosa* are associated with a threefold higher rate of mortality, a nine fold higher rate of secondary bacteremia, a twofold increase in the length of hospital stay, and a considerable increase in cost .[33]The increasing resistance rates of *P aeruginosa* strains to several antibiotics are expanding globally. In the United States, according to the NNIS system, 37% of the isolates were found resistant to ciprofloxacin, 32% to imipenem, and 22% to ceftazidime.[31] Relevant figures for intensive care unit (ICU) isolates derived from Europe are even worse, because from 1990 to 1999, resistance to aminoglycosides reached 37% to 70%, resistance to ceftazidime reached 57%, resistance to piperacillin-tazobactam reached 53%, resistance to ciprofloxacin reached 56%, and resistance to imipenem reached 52%.[34] Global resistance surveillance in the MYSTIC program from 2000 to 2006 found resistance patterns of *Pseudomonas aeruginosa* strains in United States 19.1% to19%, Northern Europe 27.8% to 31.3%, Southern Europe 35.4% to 27.6%, and in Eastern Europe 48% to 35.8%.[35]

Our study results are in agreement with reports from other countries that have shown high antimicrobial resistance rates in ICU patients. [36, 37, 38] In fact, our ICU shows much higher resistance rates. Extended use of inappropriate antimicrobials has led to the emergence of MDR species, which are extremely difficult to treat.[39] These findings also suggest other possibilities for our high resistance rates, such as inappropriate, uncontrolled empiric therapy or cross acquisition of resistance rather than the development of natural resistance. These reasons justify the need for establishing prompt infection control strategies in hospitals with special consideration in critical patient care areas. We must seriously consider implementation of the strategies recommended by the Centers for Disease Control and Prevention to prevent antimicrobial resistance in health care settings, which are: prevent infection, diagnose and treat infection effectively, use antimicrobials wisely and prevent transmission of infection.

This single center study data may not reflect antibiotic susceptibility from whole of the country. As a consequence, a multi-site study is advised to compare and contrast from other hospitals. All of the isolates may not represent actual infection from patients. A positive culture report does not mean that patient is suffering from infection and antibiotics are required.

Systemic or local antibiotics should only be prescribed after clinical correlation. For contaminants and for colonizers with some exceptions, antimicrobial agents are not required.

Conclusion

This study presents a general overview of the incidence and antimicrobial resistance of bacteria isolated from our ICU and IMCU from 2011 to 2012.The study also shows the emergence and rates of MDR organisms during the year, and emphasizes the importance of timely clinical and bacteriological monitoring among patients in a critical care situation. Infection control programs should focus on preventing infections in patients who are at highest risk of infection because of exposure to certain procedures and medical devices.

Frequent hand washing and good aseptic technique should be reinforced for all health care personnel. All healthcare settings must have a comprehensive hand hygiene program and policies and procedures in place. Excellent antibiotic policy and infection control implementation are important priorities for the critical patient areas. The microbiology laboratory should be involved in all aspects of the infection control program. Particularly important are its roles in the hospital's infection surveillance system and in assisting the infection control program to effectively and efficiently use laboratory services for epidemiologic purposes. Continued efforts are needed to develop new antimicrobial agents against MDR-organisms and to assess the currently available agents.

References

1. Mark E Jones, Deborah C Draghi, Clyde Thornsberry *et al*. Emerging resistance among bacterial pathogens in the intensive care unit – a European and North American Surveillance study (2000–2002). Annals of Clinical Microbiology and Antimicrobials 2004, 3:14
2. Nermin K Saeed, Abdulmageed M Kambal, Noura A El-Khizzi. Antimicrobial-resistant bacteria in general intensive care unit in Saudi Arabia. Saudi Med J 2010; Vol. 31 (12): 1341-49.
3. Spellberg B, Guidos R, Gilbert D *et al*. The epidemic of antibiotic resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Infect Control Hosp Epidemiol 2008; 29:271-74.
4. CDC NNIS system: National nosocomial infections surveillance (NNIS) system report, data summary from January 1992-April issued August 2001. Amer J Infect Contr 2001, 29:400-421. Correction 2002, 30:74
5. Hakan Hanberger, Jose-Angel Garcia-Rodriguez, Miguel Gobernado *et al*. Antibiotic Susceptibility among aerobic Gram-negative Bacilli in Intensive Care Units in 5 European Countries. JAMA 1999;281:67-71
6. O Fallon E, Pop-Vicas A, D Agata E. The emerging threat of multi-drug resistant gram-negative organisms in long term care facilities. J Gerontal A Biol Sci Med Sci 2009;64:138-41.
7. Carlet J, Ben Ali A, Chalfine A. Epidemiology and control of antibiotic resistance in intensive care unit. Curr Opin Infect Dis 2004; 17:309-16.
8. Ntagiopoulos PG, Paramythiotou E, Antoniadou A, *et al*. Impact of an antibiotic restriction policy on the antibiotic resistance patterns of gram-negative microorganisms in an intensive care unit in Greece. Int J Antimicrob Agents 2007; 30:360–5.
9. R. Falgas ME, Koletsi PK, Bliziotis IA. The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa J Med Microbiol 2006*; 55:1619-1629.
10. Chuon-Yi Lee, Po-Yen Chen, Fang-Liang Huang *et al*. Microbiologic spectrum and susceptibility pattern of clinical isolates from pediatric intensive care unit in a single medical center – 6 years experience. J Microbiol Immunol Infect 2009;42:160-165.
11. Giovanni Battis ta Oris, Marco Falcone, Mario Venditti. Surviellance and management of Multidrug resistant microorganisms. Expert Rev Anti Infect Ther. 2011;9(8):653-79.
12. El Kholy A, Baseem H, Hall GS, et al: Antimicrobial resistance in Cairo, Egypt 1999-2000: A survey of five hospitals. J Antimicrob Chemother 51:625-630, 2003)
13. Cepeda JA, Whitehouse T, Cooper B, *et al*: Isolation of patients in single rooms or cohorts to reduce spread of MRSA in intensive-care units: Prospective two-centre study. Lancet 365:295-304, 2005)
14. Giovanni Battis Orsi, Marco Falcone, Mario Venditti. Surviellance and Management of Multidrug-resistant Microorganisms. Expert Rev Anti Infect Ther. 2011;9(8):653-679.
15. Burton DC, Edwards JR, Horan TC, Jernigan JA, Fridkin SK. Methicillin-resistant *Staphylococcus aureus* central line-associated bloodstream infections in US intensive care units, 1997–2007. JAMA 2009; 301:727–36.
16. Rupp ME, Fey PD. *Staphylococcus epidermidis* and other coagulase negative staphylococci. In: *Principles and Practice of Infectious Diseases. 7th Edition*. Mandell GP, Bennett JE, Dolin R (Eds). Churchill Livingstone Elsevier, PA, USA, 2579–2589 (2010).
17. Hossam M. Ashour and Amany El-Sharif. Microbial Spectrum and Antibiotic Susceptibility Profile of Gram-Positive Aerobic Bacteria Isolated From Cancer Patients. Journal of Clinical of Oncology.Vol 25 (36), 5763-69, 2007
18. Seifert H, Baginski R, Schulze A, Pulverer G: Antimicrobial susceptibility of *Acinetobacter* species. Antimicrob Agents Chemother 1993; 37: 750–753.
19. Horan TC, Gaynes RP. Surveillance of nosocomial infections. In: Mayhall CG, editor. Hospital epidemiology and infection control. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 1659–702.

20. Cisneros JM, Reyes MJ, Pacho´n J, *et al*. Bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical findings and prognostic features. *Clin Infect Dis.* 1996;22:1026-1032.
21. Cisneros JM, and J Rodriguez-Bano. 2002. Nosocomial bacteremia due to Acinetobacter baumannii: epidemiology, clinical features and treatment. Clin. Microbiol. Infect. 8:687–693.
22. Amyes SGB, and HK Young. 1996. Mechanisms of antibiotic resistance in Acinetobacter spp.—genetics of resistance, p. 185–223. In E. Bergogne- Berezin, ML Jolly-Guillou, and KJ Towner (ed.), Acinetobacter: microbiology, epidemiology, infections, management. CRC Press, Inc., New York,N.Y.
23. Tankovic, J, P Legrand, GDe Gatines, V. Chemineau, C Brun-Buisson, and J Duval. 1994. Characterization of a hospital outbreak of imipenemresistant Acinetobacter baumannii by phenotypic and genotypic typing methods. J. Clin. Microbiol. 32:2677–2681.
24. Siegel JD, Rhinehart E, Jackson M, *et al*. 2007 guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings. Available at: www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf.
25. Wilks M, Wilson A, Warwick S *et al*. Healthcare Infection Control Practices Advisory Committee. Control of an outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus colonization and infection in an intensive care unit (ICU) without closing the ICU or placing patients in isolation. Infect Control Hosp Epidemiol 2006;27:654–8.
26. Jeong SH, Bae IK, Kwon SB, *et al*. Investigation of a nosocomial outbreak of Acinetobacter baumannii producing PER-1 extended-spectrum beta-lactamase in an intensive care unit. J Hosp Infect 2005;59:242–8.
27. Pimentel JD, Low J, Styles K, et al. Control of an outbreak of multi-drug-resistant Acinetobacter baumannii in an intensive care unit and a surgical ward. J Hosp Infect 2005;59:249–53.
28. Hsueh PR, Liu YC, Yang D, Yan JJ, *et al*. Multicenter surveillance of antimicrobial resistance of major bacterial pathogens in intensive care units in 2000 in Taiwan. Microb Drug Resist. 2001; 7: 373-82.
29. Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement M100-S19. Clinical and Laboratory Standards Institute, Wayne PA.
30. Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill. 13, pii19044 (2008)
31. Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1992 through June, 2003, issued August 2003. Available at: www.cdc.gov/ncidod/dhqp/pdf/nnis/2003NNISReport_AJIC.PDF.
32. Patzer JA, Dzierzanowska D. Increase of imepenem resistance among Pseudomonas aeruginosa isolates from a Polish paediatric hospital (1993-2002). Int J Antimicrob Agents.2007; 29:153-8.
33. Carmeli Y, Troillet N, Eliopoulos G, *et al*. Emergence of antibiotic-resistant Pseudomonas aeruginosa: comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother 1999;43(6):1379–82.
34 Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. Clin Microbiol Infect 2005;11(4):17–32.
35. Helen Giamarellou, Kyriaki Kanellakopoulou. Current Therapies for Pseudomonas Aeruginosa. Crit Care Clin 24 (2008) 261–278. available at: http://www.mystic-data.org
36. Fridkin SK: Increasing prevalence of antimicrobial resistance in intensive care units. Crit Care Med 2001; 29(4 suppl) N64–N68. Review.
37. Intensive Care Antimicrobial Resistance Epidemiology (ICARE) surveillance report data summary from January 1996 through December 1997: a report from the National Nosocomial Infections surveillance (NNIS) systems. Am J Infect Control 1999; 27: 279–284.
38. Esposito S, Leone S. Antimicrobial treatment for intensive care unit (ICU) infections including the role of the infectious disease specialist. Int J Antimicrob Agents 2007; 29:494–500.
39. Lockart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, Quinn JP, Doern GV: Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. J Clin Microbiol 2007; 45: 3352–3359