

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL
ISOLATES IN THE INTENSIVE CARE UNIT OF AL-ANSAR HOSPITAL,
SAUDI ARABIA

Ziab Zaakey Al-Ahmadey

Al-Ansar hospital, General Directorate of
Health Affairs in Medina, Ministry of
Health, Medina, Saudi Arabia

Sahar Ali Mohamed

Microbiology and Immunology
Department, Faculty of Medicine
Menoufiya University, Egypt

ABSTRACT

Nosocomial infections are becoming difficult to treat due to the increasing trend of antibiotics resistance, especially the critically ill patients in the intensive care unit (ICU). Antibiotics resistant Gram positive and negative bacteria cause hospital acquired infections. To identify the prevalence of predominant bacterial infections and to evaluate the antibiotic susceptibility testing of bacterial pathogens in the ICU of al-ansar hospital, Medina. During a 12 months period (from January to December 2013), a total of 1226 isolates were collected from various samples such as sputum (32%), blood (25%), urine (24%), and others (19%). All bacteria were identified by standard microbiological methods, and Microscan, antibiotic sensitivity was performed using disk diffusion technique according to CLSI guidelines and ESBLs confirmation was done by double disk diffusion method. The most common isolates were *Pseudomonas aeruginosa* (16.3%), followed by *Escherichia coli* (13.6%), *Acinetobacter baumannii* (10.4%), *Klebsiella pneumonia* (8.5%), and *Staphylococcus aureus* (6.3%). Both Gram-positive and-negative isolates expressed resistance to most of the penicillin and cephalosporins tested. *Ps. aeruginosa* was highly sensitive to piperacillin/tazobactam, imipenem, and amikacin and showed high degree of resistance to cefotaxime (90%) and ceftriaxone (85%). *E. coli* showed resistance to tetracycline (86%), piperacillin (78%), and co-trimoxazole (75%). *A. baumannii* was highly resistance to third generation of cephalosporin. High frequencies of multi-drug resistant bacteria in ICU. High rates of the ESBLs of *K. pneumonia* and *E. coli* were observed (49% and 40%, respectively). VRE made up 14% of enterococci and MRSA made up 43% of *Staphylococci* isolates.

Keywords: Intensive care unit, Antibiotic susceptibility testing, Bacterial resistance, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*.

INTRODUCTION

Antibiotic resistance is a major worldwide problem in the intensive care unit (ICU). Nosocomial infections are associated with increase in prolonged hospitalization, long duration of antibiotic usage, and mortality of infected patients was more than twice than non-infected patients (1). Rate of nosocomial infections range from 5-30% among ICU patients. The infections occur 5-10 times more often in the ICU patients than other hospitalized (2 & 3). Septicemia account for 19% of the total ICU infections, third after the urinary and respiratory infections (4). These strain are often resistance to many antibiotics, because of selective pressure due to the excessive use of broad spectrum antibiotics (3). Extended-spectrum β -lactamases (ESBLs) were first identified in the early 1980s; since then, ESBLs have been identified worldwide and have been found a number of different organisms, included *E. coli*, *K. pneumonia*,

Salmonella and *Proteus mirabilis*. Gram positive organisms are the most cause, but Gram negative carry higher risk of sepsis, septic shock and death (5).

The aim of this study was to determine the frequency of bacteria isolated from ICU patient at al-ansar hospital, Medina, Saudi Arabia and to determine the antibiotic susceptibility pattern of the causative organisms in one year.

MATERIALS AND METHODS

Between January to December 2013, a total of 1226 isolates were recovered from various clinical specimens obtained from patient who were hospitalized in the ICU of al-ansar hospital, Medina. Microbiological cultures for clinical samples comprised blood, urine, sputum and others were done. Cultures were processed using standard microbiological methods. For the blood cultures, the Bactec 9120 blood culture instrument (Becton Dickinson, Baltimore, Md., USA) was used. The blood culture bottles were incubated in the Bactec system as recommended by the manufacturer for seven days. When the positive blood bottle, three drops of blood culture samples taken up with sterile syringe and were inoculated onto blood agar, chocolate agar and Macconkey agar (Hi-Media, Mumbai, India). All the plates were incubated for 24h at 35°C aerobically overnight (5). Urine samples were cultured on blood agar, and CLED agar (Hi-Media, Mumbai, India), sputum samples were cultured on blood agar, chocolate agar, and Macconkey agar and also others samples. All the cultures were incubated aerobically (6). Identification of the bacteria was carried out based on Gram staining, standard biochemical tests and Microscan WalkAway system (Dade Behring, West Sacramento, CA) using NBC42 panels for Gram negative bacteria and PBC28 panel for Gram positive bacteria. After identification, all isolates were subcultured on Muller Hinton agar (MHA). The organisms were suspended in saline to turbidity 0.5 McFarland standards. A swab of the cell suspension was then spread in three directions on entire surface of MHA plate, and left for 15 min to allow moisture absorption at room temperature before applying the multi-disk on the agar. The agar plates were then incubated at 35°C for 18-24 h. *E. coli* (ATCC 25923) and *S. aureus* (ATCC 25922) were used as controls (6). The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (7).

Antibiotic susceptibility testing for Gram-positive bacteria included ampicillin, ampicillin/sulbactam, augmentin, cefotriaxone, clindamycin, cefazolin, ciprofloxacin, erythromycin, gentamicin, levofloxacin, linzolid, penicillin, rifampicin, synergid, co-trimoxazole, tetracycline, and vancomycin. For the Gram-negative bacteria, the antibiotics tested were ampicillin, ampicillin/sulbactam, amikacin, augmentin, cefotriaxone, ceftazidime, cephalothin, cefazolin, ciprofloxacin, cefepime, cefuroxime, tobramycin, gentamicin, imipenem, levofloxacin, piperacillin, piperacillin/tazobactam, and co-trimoxazole for *in vitro* susceptibility of bacterial isolates to these antibiotics.

ESBLs confirmation was done by double disk diffusion method as follow: ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) discs were placed on MHA plate on which a 0.5 McFarland of test organism was swabbed. Organism was considered as ESBL producer if there was ≥ 5 mm increase in zone diameter of ceftazidime/clavulanate disc and that of ceftazidime disc alone (8).

RESULTS

The widespread use of the broad-spectrum antibiotics, especially in ICUs has led to the emergence of antibiotic-resistant strains of many organisms. Samples consisting of sputum (32%), blood (25%), urine (24%) and others (19%) were collected from patients (Figure 1). *Ps. aeruginosa* 200 (16.3%) was the most frequently isolated bacteria, followed by *E. coli* 167 (13.7%), *A. baumannii* 128 (10.5%), *K. pneumonia* 105 (8.5%), *S. aureus* 77 (6.3%), and others species (44.7%). *E. coli* was the commonest Gram negative bacilli grown in urine, whereas *Ps. aeruginosa* was the most common organism in blood and *A. baumannii* was the most organism in sputum (respiratory secretions) (Table 1 and Table 2).

Table 1&2 also shows the bacteria isolated from our patients. Of the 1226 isolates, 78% (959/1226) were Gram-negative bacilli and 22% (267/1226) were Gram-positive cocci. In the Gram-positive cocci, *S. aureus* was the leading microorganism, followed by *S. epidermidis*. In the Gram-negative bacilli, *Ps. aeruginosa* was most often encountered, followed by *E. coli*, *A. baumannii*, and *K. pneumonia*. The most common source for the gram positive organism was from blood while the most common source for the gram negative organism was from sputum and urine.

Table 3 shows the antibiotic susceptibilities of Gram-positive bacteria. No Methicillin-sensitive *S. aureus* showed any resistant to linzolid, rifampicin, synergid, or vancomycin, while the highest resistance rate were revealed to be for augmentin (89%), penicillin (89%), erythromycin (46%), and tetracycline (30%). Thirty-five percent (43/120) of the cases with *S. aureus* was oxacillin-resistant. The highest resistance rate for *S. epidermidis* was for ampicillin/sulbactam (25%), ampicillin (25%), augmentin (25%), cefotriaxone (25%), ciprofloxacin (25%), and cefazoline (60%). GBS (*Streptococci agalactiae*) and GAS (*Streptococci pyogens*) were sensitive to most of the antibiotics, including ampicillin, clindamycin, levofloxacin, linzolid, penicillin, and vancomycin. Vancomycin Resistance Enterococci (VRE) made up 14% (3/21) of all enterococci spp. (mostly *E. faecium*), 2% *E. faecalis* was resistant to vancomycin, while 33% *E. faecium* was vancomycin-resistant. However, *E. faecium* showed no resistance to linzolid or synergid. *S. pneumoniae* and *S. viridans* were susceptible to cefotriaxone, penicillin, and vancomycin.

Table 4 shows the antibiotic susceptibilities of Gram-negative bacteria. For *proteus* spp., *Klebsiella* spp., *Citrobacter* spp., *Salmonella* spp., and *Serratia* spp., strains were sensitive to multiple antibiotics. *Acintobacter* spp., *Enterobacter aerogenes* and *Enterobacter cloacae* were sensitive to gentamicin and imipenem. *Ps. aeruginosa* was most commonly sensitive to piperacillin/tazobactam (85%), imipenem (82%), and piperacillin (78%), *E. coli* was sensitive to imipenem (96%), amikacin (90%), piperacillin/tazobactam (75%), and ceftazidime (60%), *A. baumannii* was sensitive to gentamicin (72%) and imipenem (75%). *K. pneumoniae* was sensitive to imipenem (97%), amikacin (80%), piperacillin/tazobactam (71%). In our study, ESBLs positivity was found to be 14%. High rates of the ESBLs of *K. pneumonia* and *E. coli* were observed (49% and 40%, respectively). The isolated bacteria showed a very high rate of resistance to the cephalosporins namely cefotriaxone, cefotaxime, cefazolin, cefuroxime, and cephalothin.

Figure 1: Source of positive culture samples isolated from the intensive care unit patients.

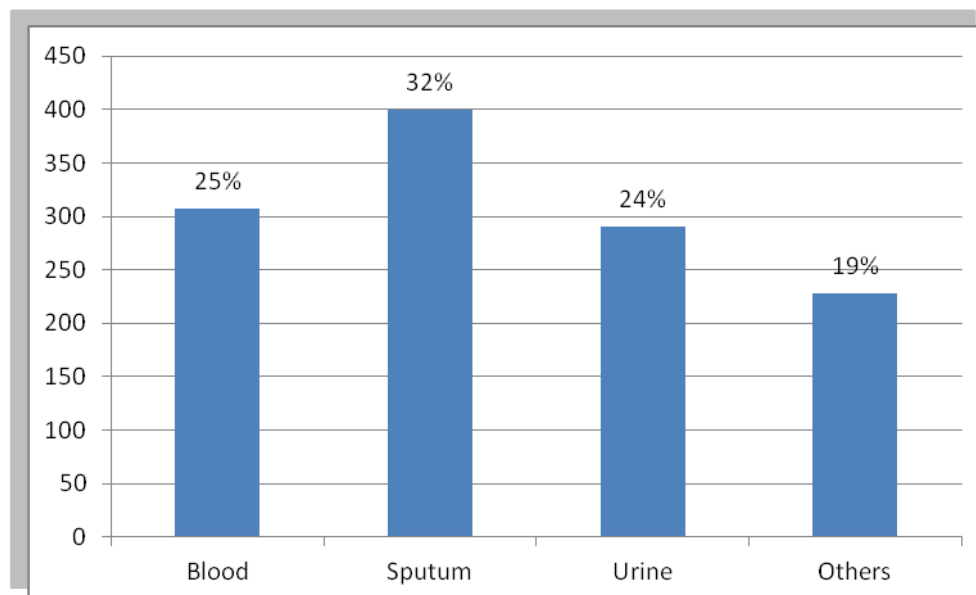


Table 1: Distribution of the Gram positive organisms isolated from ICU patient according to their source.

Organisms	Specimens				Total
	Blood	Sputum	Urine	Others	
<i>S. aureus</i>	18	22	3	34	77
<i>MRSA</i>	10	11	1	21	43
<i>S. epidermidis</i>	44	-	-	-	44
<i>GAS (S. pyogens)</i>	1	0	18	2	21
<i>GBS (S. agalactiae)</i>	1	0	0	9	10
<i>S. pneumonia</i>	4	2	0	1	7
<i>S. viridians</i>	18	-	-	-	18
<i>E. faecalis</i>	17	2	20	2	41
<i>E. faecium</i>	4	0	2	0	6
Total	117	37	44	69	267

Table 2: Distribution of the Gram negative organisms isolated from ICU patient according to their source.

Organisms	Specimens				Total
	Blood	Sputum	Urine	Others	
<i>E. coli</i>	18	26	111	12	167
<i>K. pneumonia</i>	25	37	32	11	105
<i>Klebsiella spp.</i>	5	22	6	12	45
<i>E. aerogenes</i>	9	30	4	12	55

<i>E. cloacae</i>	6	29	4	4	43
<i>P. mirabilis</i>	4	11	26	10	51
<i>Proteus spp.</i>	0	1	5	5	11
<i>Citrobacter spp.</i>	8	19	13	6	46
<i>Ps. Aeruginosa</i>	55	67	32	46	200
<i>Pseudomonas spp.</i>	3	9	1	8	21
<i>A. baumannii</i>	39	68	6	15	128
<i>Acintobacter spp.</i>	4	9	0	1	14
<i>Salmonella spp.</i>	3	0	0	3	6
<i>Serratia spp.</i>	6	17	0	4	27
<i>Providencia spp.</i>	5	18	7	10	40
Total	190	363	247	159	959

Table 3: Antibacterial susceptibility pattern of Gram-positive bacteria isolated from patients in intensive care unit wards.

Organisms	No of isolates	A/S	AM	AUG	CRO	CD	CFZ	CIP	E	GM	LUX	LZD	P	RIF	SYN	TS	TE	VA
<i>S. aureus</i>	77	98	11	98	92	87	88	93	54	81	78	100	11	100	100	98	70	100
<i>MRSA</i>	43	0	0	0	0	0	0	26	0	47	91	100	0	83	100	100	6	100
<i>S. epidermidis</i>	44	25	25	25	25	57	40	25	40	90	64	98	0	85	98	80	75	98
<i>S. agalactiae</i>	21	-	90	-	-	75	-	-	-	-	100	100	88	-	-	-	-	100
<i>S. pyogens</i>	10	90	90	100	100	60	90	90	80	40	90	100	90	-	-	50	60	100
<i>S. pneumonia</i>	7	-	86	-	100	-	-	86	71	43	-	-	100	-	-	29	-	100
<i>S. viridians</i>	18	-	94	100	100	56	94	89	83	94	89	100	94	-	-	28	72	100
<i>E. faecalis</i>	41	-	80	-	-	-	-	22	10	22	22	88	76	76	0	-	5	98
<i>E. faecium</i>	6	-	0	-	-	-	-	0	0	-	0	100	0	50	100	-	0	67

A/S, Ampicillin/sulbactam; AM, Ampicillin; AUG, Augmentin; CRO, Cefotriaxone; CD, Clindamycin; CFZ, Cefazolin; CIP, Ciprofloxacin; E, erythromycin; GM, Gentamicin; LUX, Levofloxacin; LZD, Linzolid; P, Penicillin; RIF, Rifampicin; SYN, Synercid; TS, Co-trimoxazole; TE, Tetracycline; VA, Vancomycin.

Table 4: Antibacterial susceptibility pattern of Gram-negative bacteria isolated from patients in intensive care unit wards.

Organisms	No of isolates	A/S	AK	AM	AUG	CRO	CAZ	CF	CXT	CFZ	CIP	CPE	CUR	GM	IMP	LVX	P/T	PIP	TS	TE	TN
<i>A. baumannii</i>	128	33	33	-	0	15	26	0	15	-	22	22	-	77	75	22	-	22	19	60	37
<i>Acinetobacter spp</i>	14	50	50	-	0	50	50	0	0	-	0	0	-	100	100	50	-	50	50	70	60
<i>E coli</i>	167	24	90	28	45	57	60	30	53	45	43	53	49	63	96	45	75	22	25	14	55
<i>K. pneumonia</i>	105	54	80	0	49	51	51	46	51	49	60	51	49	66	97	66	71	34	63	54	57
<i>Klebsiella spp</i>	45	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	-	100
<i>Ps. aeruginosa</i>	200	-	70	-	-	15	65	-	10	-	65	65	-	58	82	68	85	78	-	-	72
<i>Pseudomonas spp</i>	21	-	67	-	-	67	100	-	67	-	67	67	-	67	67	67	100	100	67	33	67
<i>E. aerogenes</i>	55	0	75	0	0	50	50	50	50	0	50	50	50	100	100	100	75	45	65	55	75
<i>E. cloacae</i>	43	0	90	0	0	45	67	0	55	10	80	75	25	100	100	100	80	45	67	55	75
<i>Citrobacter spp.</i>	46	50	100	0	0	50	100	50	75	50	100	100	75	100	100	100	100	75	50	50	100
<i>P. mirabilis</i>	51	50	100	17	67	100	100	83	100	83	50	100	67	67	100	67	100	67	50	0	83
<i>Proteus spp</i>	11	100	100	100	100	100	100	100	75	75	100	75	75	100	100	100	100	100	75	75	100
<i>Providencia spp</i>	40	0	67	0	0	50	0	0	33	0	17	50	0	0	100	17	85	67	67	0	0
<i>Serratia spp</i>	27	0	100	0	0	100	100	0	100	-	100	100	0	100	100	100	100	100	100	33	100
<i>Salmonella spp</i>	6	75	-	75	75	100	100	-	100	-	100	100	-	-	100	100	75	75	100	75	-

A/S, Ampicillin/sulbactam; AK, Amikacin; AM, Ampicillin; AUG, Augmentin; CRO, Ceftriaxone; CAZ, Ceftazidime; CF, Cephalothin; CXT, Cefotaxime; CFZ, Cefazolin; CIP, Ciprofloxacin; CPE, Cefepime; CUR, Cefuroxime; GM, Gentamicin; IMP, Imipenem; LVX, Levofloxacin; P/T, Piperacillin/tazobactam; PIP, Piperacillin; TS, Co-trimoxazole; TE, Tetracycline; TN, Tobramycin.

DISCUSSION

It is well known that multidrug resistant (MDR) bacteria are becoming increasingly prevalent in ICU environment as a result of extensive use of antibiotics. All Gram-negative bacteria isolates showed high frequency of resistance to multiple antibiotics but maximum resistance was observed in *A. baumannii*. The present study indicates that *E. coli* is still the most common cause of urinary tract infection. This finding is consistent with the other studies from Korea and Iran (9 & 10).

The most common Gram-positive bacteria in this study were *S. aureus* (29%) and followed by *S. epidermidis* (16.5%), together both formed approximately 10% of the total isolates. Similar studies, coagulase negative staphylococcus followed by *S. aureus* comprised the most prevalent bacteria isolated from blood (11&12). Oxacillin resistant staphylococcus spp. are an increasing global problem in nosocomial infections (13 &14). Among the 120 *S. aureus* isolates, forty-three isolates were methicillin-resistant strains (MRSA), and all were sensitive to vancomycin. In this study, resistance rate for MRSA isolates were high of tetracycline, ciprofloxacin, gentamicin, and rifampicin (94%, 74%, 53%, and 12%, respectively). This is in agreement with another study performed in Canada (15). *Enterococcus* spp., *S. epidermidis*, and MRSA isolates were also resistant to ciprofloxacin and levofloxacin. This finding is related to the most probably due to extensive usage of fluoroquinolones antibiotics in the ICU at al-ansar hospital. Our study also showed that VRE made up 14% (3/21) of all enterococci (mostly *E. faecium*). Previous data have suggested that *E. faecium* is the predominant genotype in North America (16). The relative low level of VRE in our ICU may reflect the relative variation due to geographical variation in susceptibility trends (17).

Our result revealed that *Ps. aeruginosa* (16.3%), *E. coli* (13.6%), *A. baumannii* (10.5%), and *K. pneumonia* (8.5%) were the predominant isolates in ICU of al-ansar Hospital. Similar findings have been observed in Saudi Arabia and United State (17& 18). A study in Saudi Arabia revealed the predominance of *Ps. aeruginosa* (20%), *A. baumannii* (19%), *K. pneumonia* (13%) (19). The most common Gram negative bacteria reported to cause infections in the ICU in the United State from 1993-2004 were *Ps. aeruginosa*, *E. coli*, *K. pneumonia*, and *E. cloacae*, but another study showed the most frequent bacteria isolated were *Ps. aeruginosa*, *E. aerogenes*, and *E. coli*.

Based on our data, *A. baumannii* and *Ps. aeruginosa* demonstrated multidrug resistance to several antibiotics. *Ps. aeruginosa* (90%), *A. baumannii* (85%), *Providencia* spp. (67%), *E. aerogenes* (50%), *K. pneumonia* (49%), and *E. coli* (47%) resistant to ceftriaxone. Imipenem was the most effective (82%) antibiotic against *Ps. aeruginosa* followed by amikacin and piperacillin (70% and 78%, respectively). *A. baumannii* isolates showed high rate of resistance to ceftriaxone, co-trimoxazole, and cefepime (85%, 81%, 78%, respectively). Unlikely, other studies demonstrated that 96%-100% *Ps. aeruginosa* and *K. pneumonia* isolated from ICU patients were resistant to ceftazidime (20 & 21). *K. pneumonia* was also multidrug resistant bacteria to the third generation cephalosporins antibiotics. *K. pneumonia* showed high rate of resistance to piperacillin, cefazolin, cefuraxime, cefepime (66%, 51%, 51%, 49%, respectively). This finding are consistent with those previously reported by other researchers (22, 16 & 17).

On the other hand, the increased incidence of Gram-negative bacteria resistance to ciprofloxacin and levofloxacin (fluoroquinolones antibiotics) especially *A. baumannii* (78%

and 78%), *E. coli* (57% and 55%), and *Ps. aeruginosa* (35% and 32%), respectively. This agrees with the work of Zhanel et al (2003) who found increase resistance to fluroquinolones antibiotics (23).

ESBL-producing microorganism are an increasing problem in ICU worldwide. In our study, we detected high rates of ESBL in *K. pneumonia* (49%) and *E. coli* (40%) by double disk diffusion test. This was in agreement with the results obtained by Al-Agamy et al., 2009, in a study also in Saudi Arabia (8). On other hand, other study in Saudi Arabia (19) reported that *E. coli* are becoming more common than ESBL-producing *Klebsiella* spp. In European countries and in the USA, the rates of ESBL positivity in *E. coli* and *K. pneumonia* isolates were lower than those of our study (24 & 25).

Our study results are in agreement with reports from our country (26) and other countries (27&28) that have shown high antimicrobial resistance rates in ICU patients. The increased incidence of multidrug resistance among ICU patients may be due to reasons, such as prior antibiotic use, long antibiotics exposure, and inadequate antibiotic therapy. Resistance to antibiotics poses a serious and growing problem, because such resistant bacteria are becoming more difficult to treat. 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. So the empirical and the indiscriminate use of antibiotics should be avoided and prompt infection control strategies in hospitals with special consideration in critical patient should be established in order to decrease the emergence and the spread of drug resistance among bacterial pathogens.

CONCLUSION

This study showed that *Ps. aeruginosa*, *E. coli*, *A. baumannii*, *K. pneumonia*, *S. aureus*, and *S. epidermidis* are the most common isolates obtained from ICU department of al-ansar hospital, KSA in one year study. Sputum specimens represented nearly 32% of all the specimens collection in the ICU. The multidrug resistant is common with *A. baumannii* and *Ps. aeruginosa*. Reduction of antimicrobial resistance is a goal of all ICU's around the world. Strict infection control measures like contact precautions and stringent adherence to hand washing practices, formulation of antibiotic policy, surveillance activities, must be applied.

REFERENCES

1. Lee NC, Chen SJ, Tang RB, Hwang BT. Neonatal Bacteremia in a Neonatal Intensive Care Unit: Analysis of Causative Organisms and Antimicrobial Susceptibility. J. Chin. Med. Assoc. 2004;67:15-20.
2. Singh A, Sen MR, Anupurba S, et al. Antibiotic sensitivity pattern of the bacteria isolated from nosocomial infections in ICU. J. Commun. Dis. 2002;34:257-63.
3. Arnoni MV, Berezin EN and Martino MDV. Risk factors for nosocomial bloodstream infection caused by multidrug resistant gram-negative bacilli in pediatrics. Brazil. J. Infect. Dis. 2007;11(2):267–271.
4. Fridkin SK. Increasing prevalence of antimicrobial resistance in intensive care units. Crit. Care. Med. 2001;29(1):64-8.
5. Ghadiri H, Vaez H, Khosravi S and Soleymani E. The Antibiotic Resistance Profiles of Bacterial Strains Isolated from Patients with Hospital-Acquired Bloodstream and Urinary Tract Infections. Crit. Care. Res. Pract. 2012; 6.

6. Radji M, Fauziah S, Aribinuko N. Antibiotic sensitivity pattern of bacterial pathogens in the intensive care unit of Fatmawati Hospital, Indonesia. *Asian. Pacif. J. Trop. Biomed.* 2011;39-42.
7. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility: Sixteenth informational supplement. Wayne, PA: CLSI; 2006, p.M100-S16.
8. Al-Agamy MHM, Shibl AM and Tawfik AF. Prevalence and molecular characterization of extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. *Ann. Saudi. Med.* 2009;29(4):253–257.
- 9-Kang CI, Chung DR, Son JS, Ko KS, Peck KR, and Song JH. “Clinical significance of nosocomial acquisition in urinary tract-related bacteremia caused by gram-negative bacilli,” *Amer. J. Infect. Cont.* 2011; 39(2):135–140.
10. Mohammadtaheri Z, Pourpaki M, Mohammadi F, Namdar R, and Masjedi MR. Surveillance of antimicrobial susceptibility among bacterial isolates from Intensive Care Unit Patients of a Tertiary-Care University Hospital in Iran: 2006–2009. *Chemother.* 2010;56(6):478–484.
11. Mitt P, Adamson V, Loivukene K et al, Epidemiology of nosocomial bloodstream infections in Estonia. *J. Hosp. Infect.* 2009;71(4):365-70,
12. Richards M, Thursky K, and Buising K. Epidemiology, prevalence, and sites of infections in intensive care units. *Sem. Resp. Crit. Care Med.* 2003; 24(1): 3–22,
13. Diekema DJ, Pfaller MA, Schmitz FJ et al. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clin. Infect. Dis.* 2001; 32(2):114-132.
14. Kumar S, Rizvi M, Vidhani S and Sharma VK. Changing face of septicemia and increasing drug resistance in blood isolates. *Ind. J. Path. Microbiol.* 2004; 47(3): 441–446.
15. Christianson S, Golding GR, Campbell J, the Canadian Nosocomial Infection Surveillance Program, Mulvey MR. Comparative Genomics of Canadian Epidemic Lineages of Methicillin-Resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* 2007;45:1904-11.
16. Deshpande LM, Fritsche TR, Moet GJ, Biedenbach DJ and Jones RN. Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. *Diagn. Microbiol. Infect. Dis.* 2007;58:163-170.
17. Al Johani SM, Akhter J, Balkhy H, El-Saed A, Younan M, Memish Z. Prevalence of antimicrobial resistance among gram negative isolates in an adult intensive care unit at a tertiary care center in Saudi Arabia. *Ann. Saudi. Med.* 2010;30:364-9.
18. Lockhart SR, Abramson MA, Beekman SE, Gallagher G, Riedel SR, Diekma DJ, et al. Antimicrobial resistance among Gram negative bacilli as causes of infections in intensive care unit patients in the united state between 1993 and 2004. *J. Clin. Microbiol.* 2007; 45:3352-59.
19. Saeed NK, Kambal AM, El-hazzi NA. Antimicrobial-resistant bacteria in a general intensive care unit in Saudi Arabia. *Saudi. Med. J.* 2010;31(12):1341-49.
20. Sofianou DC, Constandinidis TC, Yannacou M, Anastasiou H, Sofianos E. Analysis of risk factors for ventilator associated pneumonia in a multidisciplinary intensive care unit. *Eur. J. Clin. Microbiol. Infect. Dis.* 2000;19:460-3.
21. Goel N, Chaudhary U, Aggarwal R, Bala K. Antibiotic sensitivity pattern of gram negative bacilli isolated from the lower respiratory tract of ventilated patients in the intensive care unit. *Ind. J. Crit. Care. Med.* 2009;13:148-51.

- 22 . Thongpiyapoom S, Narong MN, Suwalak N, Jamulitrat S, Intaraksa P, Boonrat J, et al. Device associated infections and patterns of antimicrobial resistance in a medical-surgical intensive care unit in a university hospital in Thailand. *J. Med. Assoc. Thai.* 2004;87:819-24.
23. Zhanel GG, Lating NM, Nicol KA, Palatni LP, Noreddin A, Hisanaga T, et al. Antibiotic activity against urinary tract infection (UTI) isolates of vancomycin-resistant enterococci (VRE): results from the 2002 North America Vancomycin Resistant Enterococci Susceptibility Study (NAVRESS). *J. Antimicrob. Chemother.* 2003; 52:382-388.
24. Winokur PL, Canton R, Casellas JM and Legakis N. Variations in the prevalence of strains expressing an extended-spectrum β -lactamase phenotype and characterization of isolates from Europe, the American and the Western Pacific region. *Clin. Infect. Dis.* 2001;32:94-103.
25. Hanberger H, Diekema D, Fluit A, Jones R, Struelens M, Spercer R and Wolff M. Surveillance of antibiotic resistance in European ICUs. *J. Hosp. Infect.* 2001; 47, 161-176.
26. Mumtaz A K. Bacterial Spectrum and Susceptibility patterns of Pathogens in ICU and IMCU of a Secondary Care Hospital in Kingdom of Saudi Arabia . *Int. J. Patho.* 2012; 10(2): 64-70
27. 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.
28. 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