European Journal of Advanced Research in Biological and Life Sciences
Vol. 1 No. 1, 2013

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

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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%), *Acintobacter 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 cefotriaxone (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 entercococci and MRSA made up 43% of Staphyloccoci isolates.

Keywords: Intensive care unit, Antibiotic susceptibility testing, Bacterial resistance, Pseudomaonas aeruginosa, Acintobacter 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 ß-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*,

Sallmonella and *Proteus mirabils*. 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, Baltmore, 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 35ºC 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, synercid, 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, synercid, 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/sulbactum (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 synercid. 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 pipracillin/tazobactam (85%), imipenem (82%), and piperacillin (78%), *E. coli* was sensitive to imipenum (96%), amikacin (90%), pipracillin/tazobactam (75%), and ceftazidime (60%), *A. baumannii* was sensitive to gentamicin (72%) and imipenum (75%). *K. pneumoniae* was sensitive to imipenum (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.

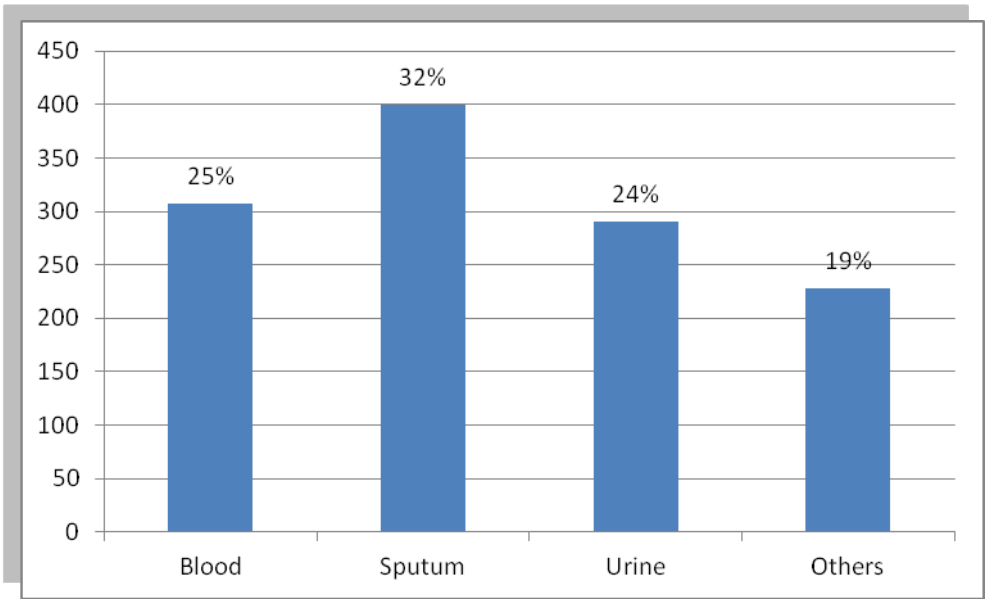

Table 1: Distribution of the Gram positive organisms isolated from ICU patient according to their source.

Organisms	Specimens				Total
	Blood	Sputum	Urine	Others	
S. aureus	18	22	3	34	77
MRSA	10	11	1	21	43
S. epidermidis	44	-	-	-	44
GAS (S. pyogens)	1	0	18	2	21
GBS (S. agalactiae)	1	0	0	9	10
S. pneumonia	4	2	0	1	7
S. viridians	18	-	-	-	18
E. faecalis	17	2	20	2	41
E. faecium	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	
E. coli	18	26	111	12	167
K. pneumonia	25	37	32	11	105
Klebsiella spp.	5	22	6	12	45
E. aerogenes	9	30	4	12	55

E. cloacae	**6**	**29**	**4**	**4**	**43**
P. mirabilis	**4**	**11**	**26**	**10**	**51**
Proteus spp.	**0**	**1**	**5**	**5**	**11**
Citrobacter spp.	**8**	**19**	**13**	**6**	**46**
Ps. Aeruginosa	**55**	**67**	**32**	**46**	**200**
Pseudomonas spp.	**3**	**9**	**1**	**8**	**21**
A. baumannii	**39**	**68**	**6**	**15**	**128**
Acintobacter spp.	**4**	**9**	**0**	**1**	**14**
Salmonella spp.	**3**	**0**	**0**	**3**	**6**
Serratia spp.	**6**	**17**	**0**	**4**	**27**
Providenca spp.	**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
S. auraus	77	98	11	98	92	87	88	93	54	81	78	100	11	100	100	98	70	100
MRSA	43	0	0	0	0	0	0	26	0	47	91	100	0	83	100	100	6	100
S. epidermidis	44	25	25	25	25	57	40	25	40	90	64	98	0	85	98	80	75	98
S. agalactiae	21	-	90	-	-	75	-	-	-	-	100	100	88	-	-	-	-	100
S. pyogens	10	90	90	100	100	60	90	90	80	40	90	100	90	-	-	50	60	100
S. pneumonia	7	-	86	-	100	-	-	86	71	43	-	-	100			29		100
S. viridians	18	-	94	100	100	56	94	89	83	94	89	100	94	-	-	28	72	100
E. feacalis	41	-	80	-	-	-	-	22	10	22	22	88	76	76	0	-	5	98
E. faecium	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 isoletes	A/S	AK	AM	AUG	CRO	CAZ	CF	CXT	CFZ	CIP	CPE	CUR	GM	IMP	LVX	P/T	PIP	TS	TE	TN
A. baumannii	128	33	33	-	0	15	26	0	15	-	22	22	-	77	75	22	-	22	19	60	37
Acinetobater spp	14	50	50	-	0	50	50	0	0	-	0	0	-	100	100	50	-	50	50	70	60
E coli	167	24	90	28	45	57	60	30	53	45	43	53	49	63	96	45	75	22	25	14	55
K. pneumonia	105	54	80	0	49	51	51	46	51	49	60	51	49	66	97	66	71	34	63	54	57
Klebsiella spp	45	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	-	100
Ps. aeruginosa	200	-	70	-	-	15	65	-	10	-	65	65	-	58	82	68	85	78	-	-	72
Pseudomonas spp	21	-	67	-	-	67	100	-	67	-	67	67	-	67	67	67	100	100	67	33	67
E. aerogenes	55	0	75	0	0	50	50	50	50	0	50	50	50	100	100	100	75	45	65	55	75
E. cloacae	43	0	90	0	0	45	67	0	55	10	80	75	25	100	100	100	80	45	67	55	75
Citrobacter spp.	46	50	100	0	0	50	100	50	75	50	100	100	75	100	100	100	100	75	50	50	100
P. mirabilis	51	50	100	17	67	100	100	83	100	83	50	100	67	67	100	67	100	67	50	0	83
Proteus spp	11	100	100	100	100	100	100	100	75	75	100	75	75	100	100	100	100	100	75	75	100
Providencia spp	40	0	67	0	0	50	0	0	33	0	17	50	0	0	100	17	85	67	67	0	0
Serretia spp	27	0	100	0	0	100	100	0	100	-	100	100	0	100	100	100	100	100	100	33	100
Salmonella spp	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, Cefotriaxone; 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. epidermides* (16.5%), together both formed approximately 10% of the total isolates. Similar studies, coagulase negative sthaphylococcus 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 (MRSAs), 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 fluroquinolones 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. cloaceai*, 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%), *Providenica 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 (fluroquinolones antibiotics) especially *A. baumanni* (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.
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CONCLUSION

This study showed that *Ps. aeruginosa*, *E. coli*, *A. baumannii*, *K. pneumonia*, *S. aureus*, and *S. epidermides* 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.

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