Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Septicemia due to MDR isolates in ICU: a therapeutic challenge

Shashi Chopra[1]* , Gomty Mahajan[2], Jaspal Kaur[3], Sheevani[4], Mukul Chopra[5], Silky Mahajan[6]

1*,4 Associate Professor, Department of microbiology, Punjab Institute of Medical Sciences, Jalandhar.
2, 3 Assistant Professor, Department of microbiology, Punjab Institute of Medical Sciences, Jalandhar.
5 Junior Resident Department of Forensic Medicine, MMIMSR, Mullana, Ambala.
6 Junior Resident Department of Pathology, ASCOMS, Jammu.

ARTICLE HISTORY

Received: 13.07.2012

Accepted: 18.07.2012

Available online: 10.11.2012

Keywords:

MRSA, ESBL, BSI, MDR

*Corresponding author:

Email : dr.shashichopra@yahoo.com
Tel : +91 9417258884

ABSTRACT

Emergence of multidrug resistant bacterial sepsis is one of the important cause of morbidity and mortality in ICU patients. An attempt was made to determine the profile and antimicrobial resistance pattern of aerobic bacterial isolates from blood cultures of ICU patients. This was a one year prospective study in the department of Microbiology, PIMS Jalandhar. A total of 850 blood samples obtained from patients suspected of sepsis were processed aerobically and antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method. The drug resistant strains were screened by Double disc synergy test for ESBL and oxacillin and cefoxitin disk diffusion method for MRSA. Blood cultures were positive in 7.64 %(65/850) samples. Gram negative organisms were isolated in 38(58.46%) of 65 samples, with Enterobacteriaceae accounted for 84.21%(*Escherichia coli* 53.12%, *Klebsiella pneumoniae* 31.25%,Salmonella typhi 9.37% and *Proteusmirabilis* 6.25%) and *Acinetobacter baumannii* 15.80%. *Stahylococcus aur*eus (64%) followed by *Enterococcus s*p. (28%) were the major Gram positive isolates and 3.08% (2/65) isolates were fungi. High level of resistance to various antibiotics was seen among Gram negative bacteria with 81.57% (31/38) of the isolates showing multi-drug resistance. MRSA isolates were 68.75%) (11/16) and ESBL producers were 73.68 % (28/38). Owing to increased prevalence of multi-drug resistant isolates in ICU, this study stresses the need for the continuous screening for antibiotic resistance in intensive care unit.

INTRODUCTION

The intensive care unit (ICU) is the epicentre of infections, mainly due to its extremely vulnerable population of critically ill patients,with impaired host defences; high risk of bacteraemia due to underlying diseases, invasive therapeutic procedures and contaminated life support equipment which could lead to sepsis or septic shock. The additional problem of multidrug-resistant pathogens boosts the adverse impact of infections in ICUs. Several factors influence the rapid spread of multidrug-resistant pathogens in the ICU, e.g., new mutations, selection of resistant strains, and suboptimal infection control.[1] Consequently, the ICU population has one of the highest occurrence rates of (nosocomial) infections (20-30% of all ICU-admissions).[2]Increasing antimicrobial resistance rates among microorganisms isolated from BSIs are a significant problem worldwide.[3] Methicillin-resistant S. aureus (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum beta-lactamase-producing Klebsiella Spp.,carbapenem-resistant enterobactericeae, P. *aeruginosa* and Acinetobacter spp. were seen more frequently in ICU patients than in non-ICU patients in many countries.[4-6] The ICU has even been described as a factory for creating, disseminating, and amplifying antimicrobial resistance.[7] The ongoing emergence of resistance in the community and hospital is considered a major threat for public health. The initial selection of antibiotics, hence, needs the knowledge about the current antibiogram in the given area. This prospective study was undertaken to determine the profile and antibiotic resistance pattern of aerobic isolates from the blood cultures of ICU patients. The aim of this study is also to provide valuable information that will aid in the decision-making and improvement in the care of patients especially in the selection of antibiotic regimen.

MATERIALS AND METHOD

During June 2010 to May 2012, 850 blood samples with strict aseptic precautions from suspected cases of bacteraemia/ septicaemia from ICU were cultured; in microbiology laboratory of PIMS, Jalandhar. A second set was also collected in all cases about an hour later from different venipuncture site. Five milliliter (ml) of blood was collected in each set into 50 ml tryptone soya broth with SPS (1:10 dilution) respectively. Bottles were then incubated at 37°C for 7 days. Subcultures on the blood agar, chocolate agar and MacConkey agar plates were done after 24 hours, 48 hours,72 hours and on 7th day. The organisms were identified by standard conventional methods of identification.[8] Antimicrobial susceptibility testing was performed by modified Kirby Bauer disc diffusion method using commercially available disc.[9]The antibiotic discs and their concentrations in µg were: beta lactams (Oxacillin1, Cefdinir5, Cephalexin30, Cefoxitin30, Cefuroxime30, Ceftriaxone30, Cefotaxime30, Ceftazidime30, Cefuroxime30, Cefpodoxime 10, Cefepime 30) 3 Carbapenems (Imipenam10, Meropenem10), Aminoglycoside (Amikacin30, Gentamicin10), Fluroquinolones (Moxifloxacin5, ciprofloxacin5), Macrolides (Erythromycin15, Azithromycin15), βlactam/ βlactamaseinhibitors (Amoxyclav20/10, Piperacillin + Tazobactam100/10, Cefoperazone + Sulbactum75/10), Others (PolymyxinB 300units,Chloramphenico1 30, Clindamycin 2, Vancomycin 30, Linezolid 30). All the antimicrobials used for the study were obtained from Hi-Media, India. The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] The strain which was resistant to 3 classes of antibiotics was considered as Multi Drug Resistant (MDR) strain. The drug resistant strains were screened for Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta Lactamases (ESBL).

MRSA

Test for detection of MRSA was done by oxacillin (1µg) and cefoxitin (30µg) disk diffusion method on Mueller Hinton agar (MHA).[11] After over night incubation at 350c, the inhibition zones of diameter less than or equal to 10 mm of oxacillin disc and less than or equal to 21 mm of cefoxitin disc, indicated MRSA. Staphylococcus aureus ATCC 25923 was used as quality control strain.

ESBL

Isolates which were resistant to azotreonam,cefpodoxime, ceftriaxone or cefotaxime as per CLSI screening criteria were taken up for further study.

Detection of ESBL was done by Double disc synergy test by using 30µg discs of azotreonam, cefpodoxime, ceftriaxone and cefotaxime placed 15 mm(edge to edge)around an amoxyclav (20µg amoxycillin + 10µg clavulanic acid)disc on inoculated MHA plate. Inoculated plates were incubated overnight at 37°C.Enhancement of the zone of inhibition between the amoxyclav disc and any one of the β lactam discs indicated the presence of ESBL. [12] Escherichia coli (ATCC 25922) and Klebsiella pneumoniae (ATCC 700603) were used as negative and positive control reference strains, respectively.

RESULTS

The present prospective analysis revealed that in a total of 850 blood cultures, 65 (7.64%) yielded mono microbial growth. The positive culture includes Gram negative bacilli 58.46% (38/65), Gram positive cocci 38.46% (25/65) and Fungi 3.08% (2/65)

Table 1: Frequency of bacterial isolates from blood cultures

Microorganism	Number	Percentage
Gram negative bacilli	(38)	(58.46%)
Escherichia coli	17	44.73
Klebsiella pneumoniae	10	26.31
Salmonella typhi	3	7.89
Proteus mirabilis	2	5.26
Acinetobacter sp.	6	15.78
Gram positive cocci	(25)	(38.46%)
Staphylococcus aureus	16	64
Enterococci sp.	7	28
CoNS	2	04
Fungi	(2)	(3.08%)
Candida albicans	2	3.08
Total	65	100

(Table-1). Amongst Gram negative bacteria Enterobacteriaceae accounted for 84.21%(Escherichia coli 53.12%, Klebsiella pneumoniae 31.25%, Salmonella typhi 9.37% and Protreus mirabilis 6.25%) and Acinetobacter sp. 15.80%.While amongst Gram positive isolates Stahylococcus aureus (64%), Enterococcus sp. (28%) and Coagulase negative staphylococcus(CoNS) (8%) were isolated. Among the isolated *Staphylococcus aureus* strains 68.7% (11/16) were MRSA . Resistance rate of Enterococci sp. to aminoglycosides and macrolides ranges from 28.5% to 59.14% (Table-2).High level of resistance was seen among Gram negative isolates with 81.57% (Escherichia coli(15), Klebsiella pneumoniae(10) and Acinetobacter(6)) isolates resistant to 3 or more drugs (multi-drug resistant).In contrast, Salmonella typhi and Proteus mirabilis showed 100% sensitivity to all the drugs. ESBL production was seen in 73.68% (28/38)(Table-2). The antimicrobial resistance pattern of resistant isolates are shown in the table number 2.

DISCUSSION

Antimicrobial resistance is a continuing serious problem in the treatment of BSIs in ICU. In the present study, isolation rate of blood culture positive cases was 7.64%, where as 4.60% was reported in another study.[13] Infection rate in ICUs have been documented to be the highest of all hospital acquired infections in large multicentre studies in the United State of America (USA) and Europe.[14,2] Gram negative organisms (58.46%) have been the more prevalent causative pathogens of septicemia in the present study than Gram positive organisms. Similar findings have also been reported by other workers (67.5%).[14] The changing etiological agents of septicemia may reflect the changing demography of septicemia in developing countries, which might be related to the geographical variations.[15]The analysis of frequency of Gram negative pathogens in current study revealed that Escherichia coli 44.73%,,*Klebsiella pneumoniae* 26.31% and *Acinetobacter baumann*ii 15.78% were the common causative organisms implicated in BSIs where as Alam MS. et al reported Acinetobacter species 31%, Salmonella typhi 24.1% and Escherichia coli 23.3% as the most common pathogens.[15] In the present study Escherichia coli(53.12%)and *Klebsiella pneumoniae* (31.25%) were the most commonly isolated Enterobacteriaceae. This is comparable to the finding of other workers, 15.17% and 14.99% respectively[16] .In the

Table 2: The antimicrobial resistance pattern of resistant isolates

Antimicrobial Agents	A	B	C	D	E
Beta Lactam	R(47.22%)	(%)	R(74.73%)	R (100%)	R (100%)
Oxacillin	11 68.75	Not tested	NT	NT	NT
Cephalexin	11 68.75		NT	NT	NT
Cefoxitin	10 62.5		NT	NT	NT
Cefuroxime	04 25		16 94.11	10 100	06 100
Ceftriaxone	04 25		08 47.05	10 100	06 100
Cefotaxime	06 62.5		12 74.58	10 100	06 100
Ceftazidime	06 62.5		09 52.94	10 100	06 100
Cefpodoxime	08 50		09 52.94	10 100	06 100
Azotrenam	NT		16 94.11	10 100	06 100
Cifdinir	NT		16 94.11	10 100	06 100
Cefepime	NT		12 74.58	10 100	06 100
Carbapenems	R (0%)	R (0%)	R (5.88%)	R (0%)	R (0%)
Meropenem	S 0	S 0	02 11.76	0 0	S 0
Imipenem	S 0	S 0	0 0	0 0	S 0
Aminoglycoside	R (18.75%)	R (28.57%)	R (3.92%)	R (66.66%)	R(83.33%)
Gentamicin*	04 25	04 57.10	02 11.76	10 100	05 83.33
Amikacin	02 12.5	S 0	S 0	0 0	05 83.33
Netlimicin	02 12.5	S 0	S 0	10 100	05 83.33
Fluroquinolones	R (6.25%)	R (0%)	R (88.23%)	R (50%)	R (100%)
Ciprofloxacin	01 6.25	S 0	15 (83.23)	04 40	06 100
Moxifloxacin	01 6.25	S 0	15 (83.23	06 60	06 100
Macrolides	R(28.12%)	R (59.14%)	Not tested	Not tested	Not tested
Erythromycin	05 31.25	04 59.14			
Azithromycin	04 25	04 59.14			
βlactam/ β lactamase inhibitors	R(33.33%)	R (0%)	R (52.93%)	R (66.66%)	R (61.10%)
Amoxyclav	16 100		16 94.11	10 100	06 100
Piperacillin+Tazobactam	S 0		02 11.76	05 50	04 66.66
Sulbactum+Cefoperazone	S 0		0 0	05 50	01 16.66
Others	R (0%)	R (0%)	R (0%)	R (0%)	R (16.66%)
Clindamycin	S 0	S 0	N T	N T	NT
Vancomycin	S 0	S 0	N T	NT	NT
Linezolid	S 0	S 0	N T	NT	NT
Polymyxin-B	NT	NT	02 (11.76)	0 0	01 16.66
Chloramphenicol	NT	NT	0 0	0 0	01 16.66

A - Staphylococcus aureus, B - Entrococci sp., C - Escherichia coli, D - Klebsiella pneumoniae, E - Acinetobacter baumannii, R - Resistant, S-Sensitive, * HLG used for Enterococcus species.

current study *Acinetobacter baumannii* was isolated in 15.76%, where as 32% was reported by other workers.[17] Reports of Acinetobacter species bacteremia are increasing especially from Asian countries and neighborhood countries of Iran such as Iraq, Kuwait, Turkey and Afghanistan.[18] An incidence of Proteus mirabilis was 5.26% in this study where as 1.33% was reported by other workers.[19]

In our study, resistant rate of Gram negative microorganisms to β lactams, aminoglycosides and fluroquinolones was 54.94%,30.78% and 47.64%,where as in another study it was reported to be 65.58%, 54.45% and 13.7% respectively. [17]. Among aminoglycosides, amikacin was a drug with 100% sensitivity to *Escherichia coli* and *Klebseilla pneumoniae*. Superiority of amikacin was also reported by others.[20,21] 81.57 % of the gram-negative isolates were multi drug resistant. During the past decades, a shift in the MDR dilemma has been noted from gram-positive to gram-negative bacteria, especially due to the scarcity of new antimicrobial agents active against resistant gram-negative microorganisms.[22] In this study, out of 38 strains of Gram negative isolates tested for ESBL production,63.15 %(24/38) were ESBL producers. Goyal A et al reported almost similar (55%) results. [23] Resistance to βlactam/ β lactamase inhibitors was 36.13% which is in accordance with the other study(32.94%). [19] Of particular concern is the emerging resistance to carbapenems (5.88 to 25%) because they are the only treatment option available in ESBL producers. Some studies have reported even higher resistance(70%) to carbapenems.[17] The

increased resistance to carbapenems may be due to wide-spread unnecessary use of carbapenems and production of various β lactamases such as AmpC β lactamases and metallo- β lactamases in gram negative isolates.[24]-Although high rates of antimicrobial resistance was observed in our study, one encouraging observation was that Salmonella typhi which is the important causes of community acquired BSIs showed 100% sensitivity to all the drugs including ampicillin, chloramphenicol and ciprofloxacin. MDR and even ESBL production have been reported in Salmonella but in our study none of the Salmonella isolate was MDR or ESBL producer.[25]

The common Gram positive isolates in our study was *Staphylococcus aureus* (64%), where as 24.1% and was reported by other workers.[26] The finding of more Gram positive isolates may reflect on better isolation of patients and hand washing precautions in ICU. Possibly for similar reasons, in Europe and USA blood culture is more likely to yield Gram positive growth.[3] The rate of MRSA infection in our study was found to be 68.75% which is in accordance with other study (63.64%) [27] but much higher than other report(20%).[28] The driving force of resistance in MRSA is cross-contamination and admission of already colonised patients to the ICU.[29] It usually arises from nosocomial infections, indiscriminate use of antibiotics, prolonged repeated hospitalization, intra venous abuse, presence of indwelling medical devices, increased age, nasal carriage, presence of open skin lesions and are often associated with high mortality. In the present study the frequency of resistance of *Staphylococcus aureus* to β lactams, aminoglycosides and fluroquinolones was 47.22%, 18.75% and6.25 % respectively, where as higher resistance was reported by other workers (58.54%,31.3%,36.8% respectively). [17] Ciprofloxacin showed the highest microbial activity(93.75%), followed by amikacin(87.5%) for Gram positive isolates. In other studies ciprofloxacin was the most effective drug for Gram positive and Gram negative bacteria (87%).[30,17] Resistance rate of Enterococci species to different group of antibiotics (aminoglycosides and macrolides) ranged from 28.57% to 59.14% where as in other study it was reported to be 33.3% to 64.7%. [15] In our study vancomycin was a highly active drug against gram positive organisms with 100% sensitivity against MRSA and Enterococci. However, it should not be expected that this activity continues for a long time as already there have been reports of Vacomycin resistant Enterococci (VRE) and Vancomycin resistant Staphylococcus aureus (VRSA) from different centres.[31,15].Fungi (Candida albicans) were isolated in 3.08% of isolates. This is similar to 2.4% reported by Shahla Latif et al.[26] Due to their immunocompromised status, patients in the ICU are at risk of invasive candidiasis.[32]The insufficient antibiotic-prescribing practices, especially the unnecessary use of broad-spectrum antibiotics, a shift in the MDR dilemma from gram-positive to gram-negative bacteria, especially due to the scarcity of new antimicrobial agents active against resistant gram-negative microorganisms together with the insufficient hospital infection prevention program are considered to be the cause of an increased incidence of BSI in the ICUs.

CONCLUSION

Bacteremia has a great impact on mortality. Therefore, blood culture should be routinely done in all acutely ill patients. In this study the frequency of MRSA (68.75%) is considerably high. Hence apart from treatment of MRSA, strict infection control practices should be adopted to curtail the spread of this "Super bug".As noted by the Infectious Diseases Society of America's "Bad Bugs, No Drugs" campaign, during the next decade, no new antimicrobial classes are expected to be developed to target some of these multidrug-resistant Gram-negative bacilli.[33]In light of emerging Gram-negative resistance combined with an empty pharmaceutical pipeline in the foreseeable future, we must make appropriate use of both newer (e.g.tigecycline, imepenem) and older (e.g., polymyxins, sulbactam) antibiotics so that therapeutic options are still available for years to come. Prompt and effective therapy requires up to date knowledge of locally prevalent organisms and ongoing surveillance for emerging antibiotic resistance. A strong infection control team approach should be preferred, including specialists, clinical microbiologist, ICU physicians and nurses.

REFERENCES

1. Marwick C, Davey P: Care bundles: the holy grail of infectious risk management in hospital? Curr Opin Infect Dis 2009, 22:364-369
2. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M: 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. JAMA 1995, 274:639-644.
3. Seifert H and Wisplinghoff H. Blood stream infections and endocarditis. In: borriello FP, Murray PR, Funkes (ed) Topley Wilson Microbiology and Microbial infection, 2005; 1(ed) 10:509526.
4. Tam VH, Chang KT, LaRocco MT et al. Prevalence, mechanisms, and risk factors of carbapenem resistance in bloodstream isolates of Pseudomonas aeruginosa. Diagn Microbiol Infect Dis 2007; 58: 309314.
5. Mehta M, Dutta P, Gupta V. Antimicrobial susceptibility pattern of blood isolates from a teaching hospital in north India. Jpn J Infect Dis 2005; 58: 174176.
6. *Mamishi S, Pourakbari B, Ashtiani MH, Hashemi FB. Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections* at Children's Medical Center, Tehran, Iran, 1996-2000. Int J Antimicrob Agents. 2005 Nov;26(5):373-9. Epub 2005 Oct 4
7. Carlet J, Ben Ali A, Tabah A, Willems V, Philippart F, Chafine A, Garrouste-Orgeas M, Misset B: Multidrug resistant infections in the ICU: mechanisms, prevention and treatment. In 25 Years of Progress and Innovation in Intensive Care Medicine. Edited by Kuhlen R, Moreno R, Ranieri VM, Rhodes A. Berlin, Germany: Medizinisch Wissenschaftliche Verlagsgesellschaft; 2007:199-211.
8. Watt B, Miles RS, Collee JG, test for identification of bacteria. 1996; 14 (ed): 131150
9. Cheesbrough M. District laboratory practical in countries. 2000; Vol (2): 132143.
10. Clinical Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; Approved standard-9thed. CLSI document M2-A9.Clinical Laboratory Standard Institute: Wayne PA; 2006
11. Brackema NM, Van TT, Monson TA, Marshall SA and

Warshuer DM. Comparison of cefoxitin and oxacillin Disc Diffusion Methods for Detection of mecA-mediated Resistance in Staphylococcus aureus in a Large-scale studt. J Clin Microbiol. 2009; 47(1):217-219.

12. Menon T, Bindu D, Kumar CPG, Nalini S, Thirunarayan MA. Comparison of double disc and three dimensional methods to screen for ESBL production in a tertiary care hospital.Indian J Med Microbiol. 2006; 24(2):117-120. 8

13. Erdem I, Ozgultekin A,Senzoz Inan A, Ozturk Engin D,Senbayrak Akcay S, Turan G,Dincer E et al.Blood stream infections in a medical-surgical intensive care unit: incidence, aetiology, antimicrobial resistance patterns of Gram-positive and Gram-negative bacteria. Clinical Microbiology and infection.2009; 15(10):943-946.)

14. Craven DE, Kunches LM, Lichtenberg DA, Kollisch NR, Barry MA,Heeren TC et al. Nosocomial infection and fatality in medical and surgical intensive care unit patients. Arch Intern Med 1988; **148:**11618.

15. Alam MS, Pillai PK, Kapur P, Pillai KK. Resistant patterns of bacteria isolated from blood stream infections at a university hospital in Delhi. J Pharm Bioall Sci 2011;3(4):525-30.

16. Mehta M, Dutta P and Gupta V.Antimicrobial susceptibility pattern of blood isolates from a teaching hospital in North India.Jpn. J. Infect. Dis.2005;58,174-176.

17. Barati M, Taher MT, Abasi R, Zadeh MM, Barati M, Shamshiri AR. Bacteriological profile and antimicrobial resistance of blood culture isolates. Iranian Journal of Clinical Infectious Diseases 2009;4(2):87-95.

18. Tien HC, Batted A, Bryce EA, Fuller J, Mulvey M, Bernard K et al. Multidrug resistant Acinetobacter infections in critically injured Canadian forces soldiers. BMC Infect Dis 2007; 7:95.

19. Qureshi M and Aziz F. Prevalence of microbial isolates in blood culture and their antimicrobial susceptibility profiles. Biomedica 2011;27:136-139.

20. Pfaller MA, Jones RN, Doern GV, Kugler K. Bacterial pathogens isolated from patients with blood stream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENETRY antimicrobial surveillance program (United states and Canada,1997). Antimicrob Agents Chemother 1998;42(7):1762-70.

21. Sader HS, Jones RN, Gales AC, Silva JB, Pignatari AC. SENETRY antimicrobial surveillance program report: Latin American and Brazilian results for 1997 through 2001. Braz J Infec Dis 2004;8(1):25-79.

22. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J: Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009, 48:1-12.

23. Goyal A, Prasad A, Ghoshal U and Prasad KN.Comparison of disk diffusion, disk potentiation & double disk synergy methods for detection of extended spectrum beta lactamases in Enterobacteriaceae. Indian J Med Res 128, August 2008, pp 209-211.

24. Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. Indian J Med Res 2009;129:707-12.

25. Nagshetty K, Channapa ST and Gaddad SM. Antimicrobial susceptibility of Salmonella typhi in India. J Infec Dev Ctries 2010;4(2):070-073.

26. Latif S, Anwar MS and Ahmad I. Bacterial pathogens responsible for blood stream infection (BSI) and Pattern of drug resistance in a tertiary care hospital of Lahore. Biomedica 2009; 25:101- 105

27. Iffat C, Naseer AC, Tayyah MM, Sultana Q. Sensitivity patterns of Staphylococcus aureus isolates from services hospital Lahore. Pak Postgrad Med J.2002;13:170-3.

28. Udaya SC, Harish BN, Umesh Kumar PM et al. Prevalence of methicillin resistant Staphylococcus aureus in JIPMER hospital. Indian J Med Microbiol 1997;15:137-138.

29. De Kraker ME, Wolkewitz M, Davey PG, Grundmann H: Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant Staphylococcus aureus bloodstream infections. Antimicrob Agents Chemother 2011, 55:1598-1605.

30. Nwadioha S. I., E. O. P. Nwokedi, E. Kashibu, M. S.Odimayoand E. E. OkworiA review of bacterial isolates in blood cultures of children with suspected septicemia in a Nigerian tertiary Hospital African Journal of Microbiology Research, 18 February 2010;4 (4):222-225.

31. Jick S. Ciprofloxacin safety in a pediatric population. Pediatr Infect Dis J1997;16:130-4.

32. Brusselaers N, Blot S, Vogelaers D: Non-blood Candida infections in the ICU. Neth J Crit Care 2011, in press.

33. Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M, Bartlett JG.Bad bugs need drugs: an update on the development pipeline from the antimicrobial availability task force of the Infectious Diseases Society of America. Clin Infect Dis 2006; 42:657-68. (Erratum in Clin Infect Dis 2006; 42:1065; comments in Clin Infect Dis 2006; 43:383-4.).