Original Research Article

Bloodstream infection associated bacterial pathogens and antibiotic resistance - ICU based pilot study

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Abstract

Background: ICU is common place of multi-drug resistant micro-organisms. The microbiological detection of bacteraemia is important for prompt management but culture and antibiotic susceptibility tests results take 3-4 days. Thus providing updated knowledge to intesivists about antibiotic susceptibility pattern is important in initiation of empiric therapy. **Methodology:** The present study was undertaken to know the microbiological profile of BSI in patients from medical and surgical ICU. Isolates were studied for their microbial characterization, antibiotic susceptibility pattern and specific antibiotic resistance mechanism. **Results:** From 150 suspected cases, 22%developed BSI. Commonest isolates were *P. aeruginosa* and *A. Baumannii which showed* ESBL and MBL production as predominant mechanism of resistance. **Conclusion:** The high prevalence of antimicrobial resistance in isolates causing BSI in ICUs warrants implementation of strict antibiotic policy and adherence to hospital infection control measures.

Key Words: Antibiotic resistance, blood stream infection, pathogens.

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INTRODUCTION

Bloodstream infections occur 2–7 times more often in intensive care unit patients than ward patients.[1,2] Bloodstream infection (BSI) is an important cause of morbidity and mortality in all critically ill patients, resulting in frequent diagnostic testing, greater prescription of antibiotics and increased length of hospitalization[3] Though blood cultures are drawn in an effort to identify the pathogen, results are often negative or even when positive, difficult to interpret. Distinguishing between true bacteraemia and a false positive culture result is important, but complicated by variety of factors in ICU like frequent invasive interventions, use of ventilators, nebulisers, etc. False positive culture reports are costly as they often lead to more diagnostic testing and more antibiotic prescriptions and increase in hospital length of stay. Moreover the results of bacteriological cultures and antibiotic susceptibility tests take 3-4 days. However microbiological detection of bacteraemia in suspected cases of BSI is important for prompt management of patient and for treatment with appropriate antibiotics.[1,4,3] Also, most of the patients are on higher antibiotics, so ICU is common place of multi-drug resistant micro-organisms.[4] Therefore providing updated knowledge to intesivists about antibiotic susceptibility pattern assumes greater importance in initiation of empiric therapy. The present study was planned to know the microbiological cause of BSI in critically ill patients from medical and surgical ICU. Isolates were studied in detail for their microbial characterization, antibiotic susceptibility pattern and specific antibiotic resistance mechanism. The findings of this study would be of great help in formulating antibiotic policy for ICU patients.

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MATERIAL AND METHODS

This was an observational prospective study undertaken in ICU of urban tertiary care teaching hospital. Patient selection: [4,5,6,7] Patients admitted to medical and surgical intensive care units over a period of one year were included the study. All clinically suspected BSI patients admitted to ICU's and those presenting with one or more of following criteria were included in the study; i.e. (fever (>38°C), tachycardia (heart rate >98/min), tachypnea (respiratory rate >24/min) leukocytosis (> 12,000/µL), hypotension (systolic BP < 90mmHg) and hypothermia (< 36 °C). Data collection for each patient included the detailed history and laboratory reports which were recorded onto the specially designed performa. Sample Collection:[8] Patients were subjected to blood collection three times. First blood sample was collected as early as possible after admission to ICU, preferably within few hours of admission and before starting or revising antibiotics. Second blood sample was collected after 72 hours and third blood sample was collected after 7 days. An additional sample was collected when clinical suspicion was persistent and no organism was isolated in previous blood cultures. Written consent was obtained from patient or patient's relative (in case of unconscious patients) for blood collection. Brain heart infusion (BHI) broth with anticoagulant SPS was used for primary inoculation. Blood Collection procedure:[9,10,11] A set of blood samples was collected by venipuncture of peripheral veins like antecubital vein and not from indwelling central line catheter. Venipuncture site was cleaned with 70% alcohol cotton swab, allowed to dry. Then cleaned with povidone-iodine and allowed to dry; site was not palpated after cleaning. 10 ml of blood was collected with sterile needle and syringe. Inoculation of blood in blood culture broth was done at bed side. Before blood collection aluminium cap on blood culture broth bottle was removed, rubber cap was disinfected with cotton swab soaked in 70% alcohol. The collected blood was directly inoculated in to blood culture broth without changing needle. The blood culture broth was agitated immediately to prevent clotting and for well mixing of blood with blood culture broth. Laboratory Processing:[12,13] Blood culture broths were incubated in CO_2 incubator at 37^0C up to 7 days. Every 24 hours broth was subjected to agitation and grossly examined for visual indicators of growth such as turbidity, color change or pellicle formation. Subcultures were done on visualization of growth indicators or otherwise on 2nd, 4th and 7th days of incubation, onto 5% sheep blood agar, chocolate agar, MacConkey agar and two Sabouraud's Dextrose Agar slants, one slant incubated at room temperature and other at 37^0C. Slants were observed daily up to 4 weeks for any growth. Gram stained smears were performed at the time of each subculture, and examined for bacteria as well as for early detection of yeast cells. Any growth on blood agar, chocolate agar and MacConkey agar was followed as per the standard protocol. Antibiotic sensitivity test was carried out by modified Kirby Bauer disk diffusion method as per CLSI guidelines. All staphylococcal isolates were tested for methicillin resistance, by cefoxitin (30µg) disk method, and all gram negative isolates were screened for ESBL production. Confirmation of ESBL production was done by betalactamase and betalactamase inhibitor combined disk test. Novel Scheme was used for identification of ESBL producers and for screening of Amp C producers, confirmation was done by modified three dimensional test.[14,15] Meropenem resistant strains of enterobacteriaceae, *P. aeruginosa, A. baumannii* were tested for metallobetalactamase production. Imipenem and meropenem combined disc test method was used.[16]

RESULTS

There were total 727 admissions in both the ICUs during one year study period. Out of these, 150 patients with clinical suspicion of BSI i.e. 108 from medical ICU and 42 from surgical ICU, were studied in detail with respect to bacterial profile of BSI, antibiotic susceptibility pattern and mechanism of resistance. From 150 suspected cases, 33 i.e.22%developed BSI. Incidence of BSI in medical ICU was observed to be18.51% (20/108) as against 30.95% (13/42) in surgical ICU. Out of total 33 isolates, commonest isolates were *P. aeruginosa* and *A. baumannii* (Table 1). Of 33 cases of BSI, 5 were diagnosed in first blood culture contributing 15% indicating primary BSI, 8 (24%) isolates were recovered from 2nd blood culture sample and 20 (61%) isolates were recovered from 3rd blood culture sample. Organisms isolated in first blood culture sample were *P. aeruginosa* and *S. aureus.* Amongst 33 isolates, 26 (79%) were bacteria and 7 (21%) were fungi. Rate of fungemia corresponds to 4.69% (7/150). In 26 bacterial isolates, gram negative bacilli showed preponderance amounting to 81% of BSI, while gram positive cocci caused 19% of BSI. Methicillin resistance was observed in 75% of *S. epidermidis* strains. But none was resistant to vancomycin, linezolid and teicoplanin (Table3). ESBL and Amp C production was predominantly seen in all isolates of *E. coli* and *K. pneumoniae*. ESBL and MBL production was predominant mechanism of resistance in *P. aeruginosa* and *A. Baumannii* (Table 4).

Table 1: Distribution of pathogens in BSI cases

Organism	Total no.(n=33)	Percent(%)
P. aeruginosa	6	18.18
A. baumannii	6	18.18
K. pneumoniae	5	15.15
E. coli	3	9.09
C. koseri	1	3.03
S. aureus	1	3.03
S. epidermidis	4	12.12
C. albicans	3	9.09
C. tropicalis	1	3.03
A. fumigatus	1	3.03
A. niger	1	3.03
S. cerevisiae	1	3.03

Table 2: Antibiotic resistance pattern of GNB

Antibiotic	*P. aeruginosa* (n=6)	*A. baumannii* (n=6)	*K. pneumonia* (n=5)	*E.coli* (n=3)	*C.koseri* (n=1)
Ampicillin	_	_	5	3	1
Amp/Clavulanic acid	_	_	5	3	1
Piperacillin	3	6	_	_	1
Pip/Tazobactam	3	4	3	3	1
Gentamicin	5	3	2	3	1
Amikacin	6	3	2	2	1
Tobramycin	6	6	_	_	_
Cefotaxime	6	6	5	3	1
Ceftazidime	4	6	5	3	1
Cefoperazone	6	5	5	3	1
Cefepime	4	5	4	3	1
Meropenem	6	6	1	2	0
Ciprofloxacin	6	5	5	3	1
Levofloxacin	5	5	3	3	1
Tmp /Sulfamethxazole	5	5	5	2	1
Tetracycline	6	6	5	3	1
Chloramphenicol	6	_	_	_	_
Polymyxin B	1	_	_	_	_
Colistin	2	_	_	_	_

Table 3: Antibiotic resistance pattern of GPC

Antibiotic	*S. aureus* (n=1)	*S. epidermidis* (n=4)
Penicillin	0	4
Oxacillin	0	3
Erythromycin	0	1
Clindamycin	0	2
Ciprofloxacin	1	3
Linezolid	0	0
Teicoplanin	0	0
Gentamicin	0	1
Amikacin	0	1
Vancomycin	0	0
Tetracycline	1	3
Trimethoprim + Sulfamethoxazole	1	4
Quinupristin-Dalfopristin	0	2
Rifampin	0	2

Table 4: Various resistance mechanisms

Organism(*no.of strains*) strains strainds)	ESBL	AmpC	MBL
P. aeruginosa (6)	4	1	5
A. baumannii (6)	2	3	3
k. pneumoniae (5)	5	5	0
E. coli (3)	3	3	0

DISCUSSION

The incidence of blood stream infection in ICU is increasing[17, 18] It has been demonstrated to vary significantly among regions, and this in part is related to blood culturing rates, and risk factor distribution in regions[17]Reported incidence of BSI in ICU ranges from as low as 0.47% to as high as 20.9%. This is most likely due to differences in population under study, duration of study and hospital setups for intensive care management.[1,18,19,20,21,22,23,24,] The present study included total of 150 patients with clinical suspicion of BSI. The subjects were studied in detail with respect to clinical features, risk factors, microbiological profile of BSI, antibiotic susceptibility and mechanism of resistance, source of BSI and prognosis. Overall reported incidence of BSI in surgical ICU is higher than in medical ICU. This is because of more number of interventions as well as large number of study population admitted belonged to various surgical faculties like general surgery, neurosurgery, orthopaedics, cardiovascular surgery and obstetrics and gynaecological surgeries.[22,25] Majority of studies throughout the world and in India have reported higher incidence of nosocomial BSI in surgical ICU. In our study the overall incidence of BSI was 4.53%, incidence of primary BSI being 0.68% and that of nosocomial BSI was 3.85%. Incidence of BSI in medical ICU was observed to be18.51% as against 30.95% in surgical ICU. Internationally CoNS, *S. aureus*, Enterococci and Candida spp. are the most commonly reported causative pathogens for CLABSI; while gram-negative bacilli account for about 20% of the infections.[1,19] However, the microorganism profile in Indian patients depicts a different picture. While CoNS and Staphylococcus species are common gram-positive microorganisms associated with BSI, gram negative organisms have shown preponderance over gram positive organisms over the last decade.[18,26] Ritu Garg *et al* and Manjula Mehta *et al* have reported *Acinetobacter* spp., *K. pneumoniae*, *P. aeruginosa* and *E. coli* as common causes of BSI in ICU.[27,28] In the present study too commonest isolates were gram negative bacteria, *P. aeruginosa* and *A. baumannii both* (18.18%), followed by *K.pneumoniae* (15.15%) and *E. coli* (9.09%) (Table 1).In our study CONS (*S. epidermidis*) was isolated in 12.12% cases. Critically ill patients treated in ICUs frequently have an infection or are prone to develop new infections. It has been observed that the total antibiotic consumption is approximately ten times greater in ICU than in general hospital wards. This leads to the emergence of multidrug resistant pathogens, posing a tough challenge to the treating physicians and intensivists as in many such cases no viable options for treatment are available.[29,30] The organisms of great concern in the hospitals in India and elsewhere are the methicillin resistant *Staphylococcus aureus* (MRSA), high level aminoglycoside resistant enterococci (HLARE), extended spectrum betalactamase (ESBL) producing enterobacteriaceae, *Pseudomonas aeruginosa* resistant to multiple antimicrobials including carbapenems and multi-drug resistant *Acinetobacter* spp.[31,3] In the present study, (Table 2) all *P. aeruginosa* isolates were resistant to meropenem, cefotaxime, tetracycline, chloramphenicol, tobramycin, cefoperazone, amikacin and ciprofloxacin. Most of them were also resistant to gentamicin and levofloxacin, ceftazidime and cefepime. Comparatively low level of resistance was seen against piperacillin, piperacillin + tazobactam combination and colistin. These findings are in correlation with Jonathan *et al*.[22] *Acinetobacter* is emerging as multi-drug resistant nosocomial pathogen, increasingly involved in hospital-acquired infections. Its prevalence is much more in ICU.[31,32,33] In the present study 33.33% of *A. baumannii* were pan drug resistant, and rest were multi-drug resistant. Our study observed multi-drug resistance in most of isolates. Similar results are increasingly being reported nationwide.[18,31,32,33] Most of Indian studies have reported increasing resistance to methicillin, especially in CONS.[18,22,24,26] In the present study 75% *S. epidermidis* were methicillin resistant. But none was vancomycin, linezolid and teicoplanin resistant. Conversely isolated strain of *S. aureus* was sensitive to Methicillin and to other antimicrobials. ESBLs are extended spectrum betalactamases capable of conferring bacterial resistance to the penicillins, first, second and third-generation cephalosporins, and aztreonam by hydrolysis of these antibiotics. AmpC are betalactamases conferring resistance not only to third-generation cephalosporins but also to betalactam + betalactamases inhibitor combinations. Presence of any of these resistance mechanism makes treatment of BSI very difficult. In India, ESBL production amongst BSI associated Enterobacteriacae organisms has been reported to the tune of 89% (R4=26, R5=31, 34=24) and co-existence of ESBL and Amp C reported in 6% isolates.[15] Present study observations indicated multiple resistance mechanisms too in gram-negative isolates. ESBL and Amp C production was seen in all isolates of *E. coli* and *K. pneumoniae*. 66.66% *P. aeruginosa* and 33.33% *A. baumannii* were ESBL producers. Treatment for Pathogens producing Metallobetalactamases that hydrolyze third-generation cephalosporins and carbapenems is a great challenge. Percentage of MBL producing non-fermenters including pseudomonas spp have been reported to be 21 to 96%. In the present study, 100% *P. aeruginosa* and *A. baumannii* were meropenem resistant. MBL production was predominant

mechanism of resistance (83.33% in *P. aeruginosa* and 50% in *A. baumannii*).

CONCLUSION

It is concluded that Gram negative bacteria are commonest causative agent in blood stream infection in ICU patients; the most common being *P. aeruginosa* and *A. baumannii.* High level of meropenem resistance was seen in gram negative bacteria i.e. 71.4%. The high prevalence of antimicrobial resistance in isolates causing BSI in ICUs calls for urgent measures to limit their continued rise and warrants implementation of strict antibiotic policy as well as adherence to hospital infection control guidelines.

REFERENCES

1. Didier Pittet, Debra Tarara and Richard P. Wenezel. Nosocomial bloodstream infection in critically ill patients, Excess length of Stay, Extra Costs, and Attributable Mortality. JAMA 1994; 271:1598-1601.
2. Leigh G Donowitz, Richard P Wenzel and John W Hoyt. High risk of Hospital-Acquired Infection in ICU patient. Crit Care Med 1982; 10:355-357.
3. Shirin Shafazand and Ann B. Weinacker. Blood Cultures in the Critical Care Unit: Improving Utilization and Yield. Chest 2002; 122:1727-1736.
4. Robert S. Munford. Severe sepsis and septic shock, Chapter 254, In: Harrison's principles of internal medicine, 16th ed. Dannis Kasper, Anthony S. Fauci, Dan L. Longo, Eugene Braunwald, Stephen L. Hauser, J. Larry Jameson. MacGraw-Hill Medical Publishing division, USA. 1996. Vol 2:1607-1612.
5. Blood stream infection definition, Australian commission on safety and quality in health care. Available at www.health.qld.gov.au/chrisp/icare/aica_bsi_def_v1 Accessed on 4/ 11/ 2007
6. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, and Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of surgical wound infections. Am J infect control 1992; 20:271-274. Available at www.cdc.gov/ncid/dhgp/nnis_pubs.html Accessed on 24/07/2007
7. Bloodstream Infection Surveillance Definitions Available at http://www.austa.gov Accessed on 25/ 10/2008
8. Michael L. Wilson. General principles of specimen collection and transport. Clin Infect Dis 1996; 22:766-777.
9. Larry G. Reimer, Michael L. Wilson and Melvin P. Weinstein. Update on Detection of Bacteremia and Fungemia. Clin Microbiol Rev 1997; 10:444-465.
10. Little JR, Murray PR, and Traynor PS. A randomized trial of povidone-iodine compared with iodine tincture for venipuncture site disinfection: effects on rates of blood culture contamination. Am J Med 1999; 107:119-125.
11. Melvin P. Weinstein. Minireview Blood culture contamination: Persisting problems and Partial Progress. J Clin Microbiol 2003;41:2275-2278.
12. Konneman's Color Atlas and Textbook of Diagnostic Microbiology 6th edition, Lippincott Williams and Wilkins.2006
13. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 18th informational supplement. CLSI document M100-18, Wayne, Pennsylvania: 2008
14. C. Rodrigues, P. Joshi, SH Jani M. Alphonse, R. Radhakrishnan and A. Mehta. Detection of betalactamases in nosocomial gram negative clinical isolates. Indian J of Med Microbiol 2004; 22:247-250.
15. S. Singhal, T. Mathur, S. Khan, DJ Upadhyay, S Chugh, R Gaind et al. Evaluation of methods for detection of AmpC betalactamase from gram negative clinical isolates from tertiary care hospitals. Indian J of Med Microbiol 2005;23:120-124
16. B. Behera, P. Mathur and A. Das and V. Sharma. An evaluation of four different phenotypic techniques for detection of metallobetalactamases producing Pseudomonas aeruginosa. Indian J of Med Microbiol 2008; 26:233-237.
17. K. B. Laupland Incidence of bloodstream infection: a review of population-based studies Clin Microbiol Infect 2013; 19: 492–500.
18. Mohd Suhail Lone, Junaid Ahmad, Dalip K. Kakru, Lubna Samad, Shagufta Roohi,Abiroo Naqash, Akeela Fatima Blood Stream Infections in Intensive Care Units. A Study fromNorth India Journal of Microbiology and Related Research / Volume 2 Number 1 / January - June 2016
19. J. Rello, M. Ricart, B. Mirelis, E. Quiontana, M. Gurgui, A. Net and G. Prats. Nosocomial bacteremia in a medical-surgical intensive care unit: epidemiologic characteristics and factors influencing mortality in 111 episodes. Intensive Care Med 1994; 20:94-98.
20. Christian Brun-Buisson, Francoise Doyon, Jean Carlet, Pirre Dellamonica,Francois Gouin, Agnes Lepoutre et al. Incidence, Risk Factors and Outcome of Severe Sepsis and Septic Shock in Adults A Multicenter Prospective Study in Intensive Care Units. JAMA 995; 274:968-974.
21. Joseph M. Darby, Peter Linden, William Pasculle and Mellissa Saul. Utilization and diagnostic yield of blood cultures in a surgical intensive care unit. Crit Care Med 1997; 25:989-994.
22. Jonathan D. Edgeworth, David F. Treacher and Susannah J. Eykyn. A 25 year study of nosocomial bacteremia in an adult intensive care unit. Crit Care Med 1999; 27:1421-1428.
23. M. Garrouste-Orgeas, S. Chevret, J. L. Mainardi, J. F. Timsit, B. Misset and J.Carlet. A one year prospective study of nosocomial bacteremia in ICU and non- ICU patients and its impact on patient outcome. J Hosp Infect 2000; 44:206-213.
24. P. Mathur, A. Kapil and B. Das. Nosocomial bacteremia in intensive care unit patients of a tertiary care center. Indian J Med Res 2005; 122:305-308.
25. Hilmar Wisplinghoff, Tammy Bischoff, Sandra M. Tallent, Harald Seifert, Richard P. Wenzel and Michael B. Edmond. Nosocomial Bloodstream infections in US Hospitals: Analysis of 24,179 Cases from a Prospective Nationwide Study. Clin Infect Dis 2004; 39-309-317.

26. V Ramasubramanian, Vivek Iyer, Sandeep Sewlikar3 and Anish Desai4Epidemiology of healthcare acquired infection – An Indian perspective on surgical site infection and catheter related blood stream infection Indian Journal of Basic and Applied Medical Research; September 2014: Vol.-3, Issue- 4, P. 46-51
27. Manjula Mehata, Prabhjyot Kaur and Jagdish Chander. Antibiotic sensitivity pattern of bacterial isolates from blood of ICU patients in a tertiary care hospital. Hospital Infection society of India CON-2007 available at http://www.gmch.nic.in/microconf/HISICON Accessed on 23/04/ 2008
28. Ritu Girg, Neelam Kaistha and Jagdish Chander. Antibiotic sensitivity pattern of bacterial isolates from blood cultures of ICU patients in tertiary care hospital of North India. Hospital Infection society of India CON-2007 available at,http://www.gmch.nic.in/microconf/HISICON Accessed on 23/04/ 2008
29. David L. Paterson and Robert A. Bonomo. Extended-Spectrum Betalactamases: a Clinical Update. Clin Microbiol Rev 2005; 18:657-686.
30. Dennis C. J. J. Bergmansa, Marc J. M. Bontena, Carlo A. Gaillardc, Frank H. van Tielb, Siebe van der Geesta, Peter W. de Leeuwa and Ellen E. Stobberingh.Indications for antibiotic use in ICU patients: a one-year prospective surveillance. J Antimicrobial Chemotherapy 1997; 39:527-553.
31. M. S. Alam, P. K. Pillai, Prem Kapur, and K. K. Pillai Resistant patterns of bacteria isolated from bloodstream infections at a university hospital in Delhi, J Pharm Bioallied Sci. 2011 Oct-Dec; 3(4): 525–530.
32. Juhi Taneja, Bibhabati Mishra, Archana Thakur, Vinita Dogra, Poonam Loomba Nosocomial blood-stream infections from extended-spectrum-beta-lactamase-producing Escherichia coli and Klebsiella pneumonia from GB Pant Hospital, New Delhi J Infect Dev Ctries 2010; 4(8):517-520.
33. Sumita Rajeevan, Syed Mustaq Ahmad and P.T.Jasmin. Study of prevalence and antimicrobial susceptibility pattern in blood isolates from a tertiary care hospital in North Kerala, India Int.J.Curr.Microbiol.App.Sci 2014; 3(4): 655-662.

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