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OCCURRENCE AND MICROBIAL PROFILE OF BLOOD STREAM INFECTION IN TERTIARY CARE HOSPITAL INDIA

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Abstract- *Background*: The epidemiology of blood stream infection (BSI) is constantly changing. BSIs are associated with the syndrome requiring admission to intensive care unit (ICU) such as sepsis and septic shock and remains one of the most important causes of morbidity and mortality. BSI prolongs patients stay in an ICU and in hospital leads to increased health care expenses. *Methods*: This laboratory based retrospective study was conducted for a period of two years (January 2011 to December 2012) in a 1470 bed tertiary care hospital in India. A total of 2999 samples were analyzed from the hospitalized patients for whom blood culture were routinely processed in the department of Microbiology Laboratory. *Results*: The present retrospective study demonstrated 966 (32.21%) culture positivity. Of the total culture positive episodes, 374 (38.81%) were GNP and 573 (59.31%) were GPP while 19 (1.96%) were *Candida spp*.. Of the total 573 GPP, 258 (45.02%) were MSSA while 228 (39.79%) were MRSA. Among 486 *S. aureus* strains isolated from BSIs, 129 (26.54%) strains were inducible clindamycin producers of which 99 (76.74%) strains were isolated from patients admitted in various ICU. All *S. aureus* strains were susceptible to linezoilid and tigecycline and vancomycin. Present study revealed that 431 (44.61%) positive culture episodes were from patients admitted in various ICU of which 239 (55.45%) were GNP and 192 (44.54%) were GPP. Maximum patients were from MICU 162 (37.58%) and NICU 153 (35.49%) followed by medicine ward and orthopedic ward. *Conclusion*: The surveillance of BSI pathogen in a hospital is important in monitoring the spectrum of microorganism that invades the blood stream and to improve efficacy of empirical treatment protocols. It helps to clinicians to recognize the emerging pathogens that may be threat to the community. Collaboration between physicians, clinical microbiologists and infectious disease consultants should produce significant positive outcome.

Keywords- Blood stream infection, multidrug resistance, ESBL, MBL, Inducible clindamycin producer *Staphylococcus aureus*

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Introduction

The epidemiology of blood stream infection (BSI) is constantly changing. BSIs are associated with the syndrome requiring admission to intensive care unit (ICU) such as sepsis and septic shock and remains one of the most important causes of morbidity and mortality. BSI prolongs patients stay in an ICU and in hospital leads to increased health care expenses [1]. Sepsis is often lethal, killing 20 to 50 percent of severely affected patients. It is the second leading cause of death among patients in non coronary ICUs and the 10th leading cause of death overall in the United States. Furthermore, sepsis substantially reduces the quality of life of those who survive [2]. Among the BSI pathogens, *Staphylococcus aureus* (*S. aureus*) is the most frequently isolated gram positive pathogen, while *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumonia*) are most frequently isolated gram negative pathogen. There has been a recent re-emergence of gram negative organisms, particularly in developing countries contributing up to 55% of community- associated BSIs [3, 4]. Gram negative organisms remain the more common BSI pathogens in developing countries. Antibiotic susceptibility pattern of common BSI isolates have also been changing overtime. The timely and appropriate use of antibiotics is at present the only approach to treat bacteremia. However, many bacterial pathogens have become resistant to routinely used antibiotics and generate a serious public health concern with economic and social implications throughout the world [4-6]. Extended-spectrum β- lactamases (ESBLs) are group of plasmid-borne enzymes with the ability to hydrolyze oxyimino β- lactams. These enzymes predominantly found in members of family *Enterobcateriaceae* mainly in *E.coli, K. pneumoniae.* ESBLs confer resistance not only to penicillins, azetreonam and cephalosporins but could also be resistant to other antibiotic classes including aminoglycosides, trimethoprime-sulfamethoxazole and quinolones. BSIs caused by ESBLs producing gram negative pathogens is of great concern due to severely limited therapeutic options and increased risk of treatment failure in patients. It is important to determine risk factors for the infections with multidrug resistant (MDR) ESBL- *E.coli, K. pneumonia* (ESBL-EK) isolates [7-10].

BSIs should be reassessed periodically because of increased antibiotic resistance and which may vary in different ICUs depending upon the antibiotic pressure in that particular health care facility. ICUs are

mainly epicenter of these infections because of extremely vulnerable population and the increased risk of becoming infected through multiple invasive therapeutic and diagnostic procedures. In the United States there have been several national programs, which have focused on both the etiology of infections and resistance patterns of nosocomial or ICU infections including the National Nosocomial Infections Surveillance (NNIS) and concluded that, it is essential that local surveillance programs to be maintained in each country's ICU setting. The local data are vital to the formulary committees as they select appropriate agents to treat infections [11-13]. We have conducted retrospective study to determine the common bacterial agents caused BSI and their antimicrobial susceptibility pattern in tertiary care hospital in India.

Materials and Methods

This laboratory based retrospective study was conducted for a period of two years (January 2011 to December 2012) in a 1470 bed tertiary care hospital in India. A total of 2999 samples were analyzed from the hospitalized patients for whom blood culture were routinely processed in the department of Microbiology Laboratory.

Data Collections

Data on sociodemographic variables such as age, sex and clinical data included immune status, underlying disease, a history of hospitalization; previous hospitalization and antimicrobial therapy, use of mechanical ventilator, presence of an indwelling catheter, the associated focal infection, and parameters for determination of disease severity were collected.

Sample Collection

Microbiological Procedures and Methods

True bacteremia, hospital-acquired bacteremia and blood culture contaminant were defined by using conventional criteria [14]. Specimens were collected, transported by standard conventional methods. Isolation and identification of microorganisms at the species level were done by standard conventional methods [15].

Antibiotic Susceptibility Tests

The Kirby- Bauer method recommended by the CLSI guidelines (2008) was used for antimicrobial susceptibility testing [18]. An ESBL-EK isolate was defined as MDR if it was resistant to at least one member of the following two classes of antibiotics: aminoglycosides (amikacin, gentamicin or netilmycin) and fluoroquinolones (ofloxacin, or ciprofloxacin) [16]. Nosocomial blood stream infections were defined according to the criteria proposed by the Centres for Disease Control and Prevention [14].

Detection of Extended Spectrum β-Lactamases (ESBLs)-Screening Test (CLSI, 2007)

Initially screening test for ESBL production was done as part of routine susceptibility testing. Two antibiotic discs, ceftazidime (30 µg) and cefotaxime (30 µg) were used for screening for ESBLs. Mueller- Hinton Agar (MHA) were prepared and inoculated with the test organism (turbidity corresponding to 0.5 McFarland's standard) to form a lawn culture. The above discs were applied on the surface of the agar. The plates were incubated at 37ºC overnight and sensitive pattern and resistant pattern were recorded by reading the zone diameter of the test organism. If a zone diameter of ≤ 22mm for Ceftazidime and ≤27 mm for cefotaxime was recorded these strain were considered "Suspicious" for ESBL production [17].

Double Disk Approximation Test (DDAT)

Bacterial suspension equivalent to 0.5 McFarland standards turbidity for testing ESBL production test were prepared. A sterile swab was dipped into standardized inoculums and the soaked swab was rotated against the upper inside wall of the tube to express excess fluid. The entire surface of the MHA was swabbed to form a lawn culture and the inoculum was allowed to dry for a minute with lid in place. With sterile forceps, Ceftazidime disk was placed on the agar plate near the centre giving a centre to centre distance of 15 mm Ceftazidime/clavulanic acid (30µg/10µg). The plates were inverted and incubated at 37ºC for 16-18 hours. Each plate was examined for enhancement of zone of inhibition for ceftazidime disk at the side facing Ceftazidime/clavulanic acid disk. If the strain was an ESBL producer, then the zone around ceftazidime disk was extended towards Ceftazidime/clavulanic acid disk. ATCC *Escherichia coli* 25922 were used as negative control and ATCC *K. pneumoniae* - 700603 was used as positive control [17-21].

Detection of Metallo β-Lactamases by Imipenem-EDTA-Double Disk Synergy Test

The IMP-EDTA combined disk test was performed as described by Lee K. *et al*. Test organisms were inoculated on to plates with MHA as recommended by the CLSI guidelines. Two 10 µg imipenem disks (Hi Media, Mumbai India) were placed on the plate, and appropriate amounts of 10 µL of 0.5 M EDTA solution were added to one of them to obtain the desired concentration (750 µg). The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16 to 18 hours of incubation at 35°C. In the combined disc test, if the increase in inhibition zone with the imipenem and EDTA disc was ≥ 7 mm than the imipenem disc alone, it was considered as MBL positive [22,23].

Results and Observations

A total of 2999 blood samples were received of that 966 (32.21%) were culture positive from the study group. 374 (38.71%) GNP and 573 (59.31%) GPP were isolated from 966 episodes of BSIs respectively. In 19 (1.96%) *Candida spp.* were culture positive [Table-1], [Fig-1]. Investigation of these positive episodes showed that 431 (44.61%) isolates were from ICU patients. Among 966 positive culture isolates, 530 (56.86%) patients were male while 436 (45.13%) were female. 904 (93.58%) blood culture positive isolates were monomicrobial while 62 (6.41%) were polymicrobial.

Table 1- Distribution of culture positive isolates from BSI

Year	Total sample received	Culture positivity	Gram- pathogen	Gram+ Pathogen	Fungus
2011	1250	496 (39.68%)	213 (42.94%)	282 (56.85%)	1 (0.2%)
2012	1749	470 (26.87%)	161 (34.25%)	291 (61.91%)	18 (3.82%)
Total	2999	966 (32.21%)	374 (38.71%)	573 (59.31%)	19 (1.96%)

Overall high frequency of GPP i.e. 59.31% was observed and year wise distribution was increasing from 56.85% to 61.91%. Overall frequency of GNP was 38.71% and year wise distribution was changed from 42.94% to 34.25% while fungus isolation is increasing from 0.20% to 3.82% [Fig-2].

Of the total 374 GNP, *Acinetobacter spp.*119 (31.81%) was commonest GNP followed by *Klebsiella pneumonia* 73 (19.51%), *E.coli*

66 (17.64%) and *Citrobacter spp.* 62 (16.57%). Of the total 374 GNP; 194 (51.87%) GNP strains were ESBL producers while 59 (15.77%) were MBL producers [Table-2]. Among 194 ESBL producers GNP, 80 (41.23%) strains were isolated from various ICU patients.

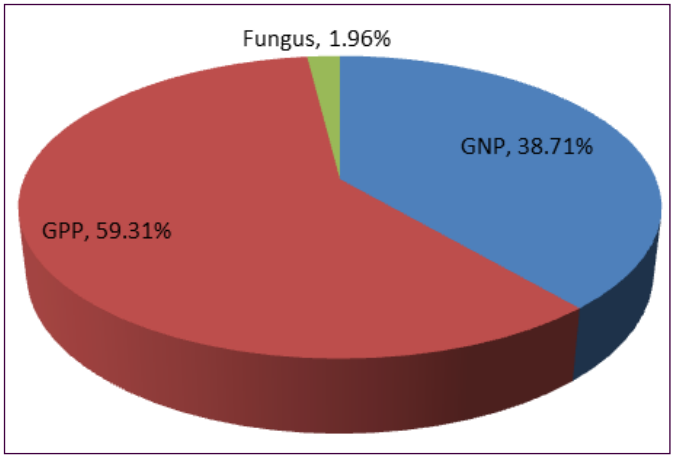

Fig. 1- Distribution of percentage frequency of positive episodes of blood culture from BSIs

Fig. 2- Year wise frequency of isolation of various pathogens from positive blood culture of BSIs

Table 2- Incidence of GNP from positive episodes of BSIs

Organism isolated	2011	2012	Total	ESBL	MBL
Acinetobacter spp.	56	63	119	62	17
Klebsiella pneumonia	42	31	73	35	18
E.coli	34	32	66	43	12
Citrobacter spp.	46	16	62	38	8
Enterobacter spp.	20	1	21	11	2
Salmonella spp.	2	12	14	1	0
Proteus spp.	9	2	11	1	0
Pseudomonas spp.	3	2	5	1	1
Others	1	2	3	2	1
Total	213	161	374 (38.71%)	194 (51.87%)	59 (15.77%)

Total 194 (51.87%) strains were ESBL producers. All ESBL strains were also tasted for antibiotics from class aminogycosides TMP/SMX and fluorquinolones and showed resistance 79%, 63%, and 83% respectively. Of the total ESBL; 30% strains were ESBL as well as MBL producers [Fig-3].

Of the total 573 GPP, 258 (45.02%) were MSSA while 228 (39.79%) were MRSA. 44 (7.6%) CONS and 36(6.28%) *Enterococcus spp.* were isolated [Table-3]. Among 486 *S. aureus* strains isolated from BSIs, 129 (26.54%) strains were inducible clindamycin producers of which 99 (76.74%) strains were isolated from patients admitted in various ICU. All *S. aureus* strains were susceptible to linezoilid and tigecycline and vancomycin. All *Enterococcus spp.* were susceptible to vancomycin and moderate susceptibility rate were recorded in erythromycin (64%) co-trimaxazole (69%).

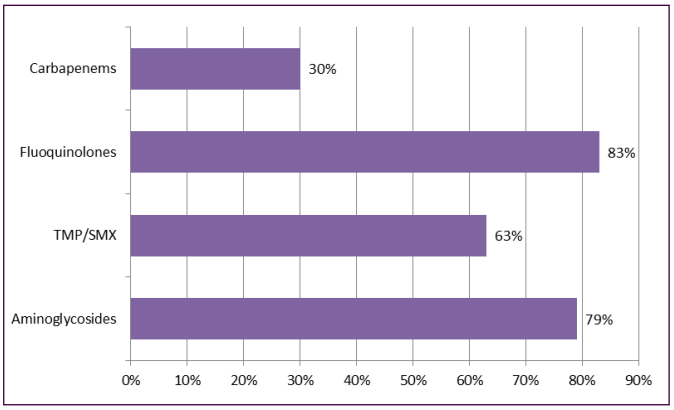

Fig. 3- Percentage Resistance of aminoglycoside and TMP/SMX and fluorquinolones class of antibiotics antimicrobial agents in ESBL producer GNP (n= 194)

Table 3- Incidence of GPP from positive episodes of BSIs

Year	MRSA	MSSA	CONS	Enterococcus spp.	Streptococcus spp.
2011	78	134	39	29	2
2012	150	124	5	7	5
Total	228	258	44	36	7

MRSA: Methiciline resistant *S. aureus*, MSSA: Methicilline sensitive *S. aureus*, CONS: coagulase negative *S. aureus*

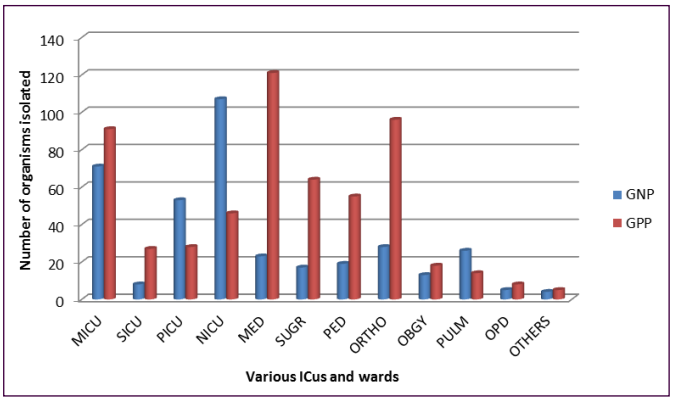

Fig. 4- Distribution of gram positive pathogen (GPP) and gram negative pathogens (GNP) from various specialty wards and ICUs

MICU: Medicine ICU, SICU: Surgery ICU, PICU: Pediatric ICU, NICU: neonatal ICU, MED: medicine, SURG: surgery, PED: pediatrics, ORTHO-orthopedic, OBGY: obstrectics and gynecology, PULM: pulmonary medicine, OPD: outdoor patients

A total of 431 (44.61%) positive culture episodes were from patients admitted in various ICU. Of which 239 (55.45%) were GNP and 192 (44.54%) were GPP. Maximum patients were from MICU 162 (37.58%) and NICU 153 (35.49%) followed by medicine ward and orthopedic ward [Fig-4].

Discussion

Information of specific etiologic agent and its antimicrobial susceptibility in BSI patient improves clinical outcome. The present retrospective study demonstrated 966 (32.21%) culture positivity. Of the total culture positive episodes, 374 (38.81%) were GNP and 573 (59.31%) were GPP while 19 (1.96%) were *Candida spp*.. Among 966 positive culture isolates, 530 (56.86%) patients were male while 436 (45.13%) were female. 904 (93.58%) blood culture positive isolates were monomicrobial etiology while 62 (6.41%) were polymicrobial. GPP showed high prevalence and common GPP were *S. aureus* i.e. 486 (84.81%).

The epidemiology of microbial pathogens causing BSI's dramatically changed over years, with a associated in-crease in antimicrobial resistance. A nationwide surveillance study conducted in 49 hospitals in USA showed a large prev-alence of GPP causing BSI's compared with GNP. However, a trend towards an increasing incidence of GNP causing BSI's has been observed more recently [24, 25]. Of the total 573 GPP, 258 (45.02%) were MSSA while 228 (39.79%) were MRSA. The MRSA rate reported in the present study is comparable to the USA rate of 52.9% described in the National Nosocomial Infections Sur-veillance (NNIS) data summary for the period 1992 - 2004 [14]. In a large prospective surveillance study, Zhanel et al reported that 22.3% of all *S. aureus* isolates in Canadian ICU's corresponded to MRSA [26]. Malacarne P et al. reported 71% percent of bacteremic episodes among patients admitted to Italian ICU's were caused by MRSA, and Hawser SP et al. reported that about 9% of the MRSA isolates had a MIC of at least 2.0 µg/mL for vancomycinin many European ICU's [27]. There was low prevalence of CONS in the present study. 36 (6.28%) CONS were isolated. Mukharjee et al. 61% and Wattal C et al. 20.3% reported CONS as predominant blood stream isolate as well as Japoni et al. 67.7%, Karlowsky JA et al. 42%, Dagnew et al. 42.3% from Northwest Ethiopia and Anug AK et al. 42% from Australia reported as common blood stream isolate [28-31].

Among 486 *S. aureus* strains isolated from BSIs, 129 (26.54%) strains were inducible clindamycin producers of which 99 (76.74%) strains were isolated from patients admitted in various ICU. All *S. aureus* strains were susceptible to linezoilid and tigecycline and vancomycin. All *Enterococcus spp.* were susceptible to vancomycin and moderate susceptibility rate were recorded in erythromycin (64%) co-trimaxazole (69%). According to data provided by the National Healthcare Safety Network (NHSN), 192 MSICU's in USA reported 578 catheter-related BSI's in 2010 [14]. MRSA has emerged as the most common hospital acquired pathogen. By 2003, MRSA represented more than 60% of all *S. au-reus* isolates in USA ICU's. From 2005 to 2008, the incidence of invasive MRSA infections declined by 34% compared to baseline rates [14]. 431 (44.61%) isolates were from ICU patients. The emergence of MDR pathogens is frequently related to extreme use of broad spectrum antimicrobial agents in critically ill patients. In the ICUs, strategies such as antimicrobial cycling and de-escalation schemes have been implemented, however the use of broad–spectrum antimicrobials in critically ill patients is considered necessary due to small margins for error in choice of therapy, where initial selection of antimicrobials covering offending pathogens is of great importance. Present study revealed that 431 (44.61%) positive culture episodes were from patients admitted in various ICU of which 239 (55.45%) were GNP and 192 (44.54%) were GPP. Maximum patients were from MICU 162 (37.58%) and NICU 153 (35.49%) followed by medicine ward and orthopedic ward.

In the present study, of the total 374 GNP, *Acinetobacter spp.*119 (31.81%) was commonest GNP followed by *Klebsiella pneumonia* 73 (19.51%), *E.coli* 66 (17.64%) and *Citrobacter spp.* 62 (16.57%). 194 (51.87%) GNP strains were ESBL producers while 59 (15.77%) were MBL producers. Among 194 ESBL producers GNP, 80 (41.23%) strains were isolated from various ICU patients. In Europe and the United States, the number of BSIs caused by ESBL-producing strains of the family *Enterobacteriaceae* is on the increase, and this trend has a significant impact on mortality rates and hospital costs. Teena Chopra et al. reported high prevalence of carbapenem and ampicillin-sulbactam resistant (CASR) *Acinetobacter baumannii* (*A. baumanii)* BSI from USA hospital, Ajay Kumar et al. reported high mortality rate in neonates from BSI caused by carbapenem resistant *A. baumanii* in India. Hanan et al. reported 60% *A. baumanii* BSI in Riyadh Saudi Arabia while Jadhav et. al reported high prevalence of *Klbesiella pneumoniae* as predominant pathogen from BSIs. [32,33]. In the present study, 1.96% Candidemia were demonstrated and all patients were admitted in ICU, associated with various risk factors include exposure to multiple antibiotics, indwelling catheters, parentaral nutrition, previous surgery. According to the surveillance data from the US Centers for Disease Control and Prevention (CDC), *Candida spp.* accounts for 12% of all hospital-acquired BSIs.

Conclusion

Development of antibiotic resistance continues to increase rapidly and the possibility that empirically prescribed treatment will ineffective is increasing. These trends can have adverse effect on clinical outcomes. The surveillance of BSI pathogen in a hospital is important in monitoring the spectrum of microorganism that invades the blood stream and to improve efficacy of empirical treatment protocols. It escort to clinicians to recognize the emerging pathogens that may be risk to the community. Collaboration between physicians, clinical microbiologists and infectious disease consultants should construct significant positive outcome.

Conflicts of Interest: None declared.

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