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Phenotypic characterization and susceptibility pattern of Gram negative bacterial isolates from cases of Central Line Associated Blood Stream Infections in a tertiary care institute.

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ABSTRACT

Background: Resistant and specially Multi Drug Resistant (MDR) Gram negative isolates from the cases of Central Line Associated Blood Stream Infections (CLABSI) are a serious challenge for the treatment to clinicians. Present study was undertaken for phenotypic characterization and susceptibility pattern of Gram negative bacterial isolates from cases of CLABSI in a tertiary care institute.Materials and Methods: This descriptive cross sectional hospital based study was conducted in a tertiary care teaching hospital over a period of one and half year from January 2012 to June 2013. CDC's CLABSI case definitions were used to label a case as CLABSI. Only culture proven cases, out of clinically suspected was included in the study for evaluation. Results: Incidence Central Line Associated Blood Stream Infections were (0.31%) with 38 culture positive cases. The overall rate of CLABSI was 14.64 per 1000 device days with (0.04) device utilization ratio. Gram negative isolates predominated (79%) over Gram positive isolates (21). Among Gram negative bacterial isolates from CLABSI, Klebsiella pneumoniae (40%) was the commonest followed by E. coli (26.66%). %). Rising trends of antimicrobial resistance for common Gram negative bacterial isolates especially multi drug resistance was noticed.Conclusion: Continuous surveillance of CLABSI and microbiological reporting of culture isolates is a need of time in the health care facility.

Key words: Central Line Associated Blood Stream Infections, Gram negative bacterial infection, Multi Drug Resistant.

INTRODUCTION

Hospitalized patients suffered by the most common complications during their hospital stay are Health care-associated infections (HAIs).Despite of significant advances in infection control policies, HAIs remain a major public health problem and a significant cause of raised morbidity and mortality among hospitalized patients.[1]Among several factors responsible for institutional morbidity and mortality Central line associated bloodstream infection (CLABSI) is a major contributing factor and consequently it is also responsible for longer hospital stay and increased expenditure on treatment.[1]Insertion of Central Venous Catheter in a chronically ill ICU admitted

patients is a common practice now a days worldwide. CVC inserted in emergency without following proper aseptic procedure, for longer duration and daily manipulation of CVC for the purpose of administration of fluids, drugs, and blood products are responsible for higher rate of CLABSI in ICU settings.[1]Higher rates of infection often leads to increased rates of antimicrobial prescription which in turn contribute to increased antimicrobial resistance.[2] Treatment of multi-drug resistant pathogens is a costly affair.[2]Data regarding the incidence, prevalence, epidemiology, aetiology and susceptibility pattern of isolates are frequently available from the resource rich countries but same is not true for resource poor countries.[3-8]Both gram positive and gram negative organisms are responsible for CLABSI. Gram positive cocci (both *Staphylococcus aureus* and the coagulase-negative staphylococci) are the leading causes of CLABSI followed by Gram negative bacilli.[9]Multidrug-resistant organisms are increasinglybeing reported.[10]

The present study was designed to study the phenotypic characterization and susceptibility of gram negative bacteria from Central Line Associated Blood Stream Infections (CLABSI) in our geographic area. The purpose of the Antimicrobial Susceptibility Test (ABST) was;

1. To guide the choice of the antimicrobial for the treatment.
2. To provide surveillance data to monitor the resistance trend.

MATERIAL AND METHOD

This descriptive cross sectional hospital based study was conducted in a tertiary care teaching hospital over a period of one and half year from January 2012 to June 2013. The study was carried out in surgical ICU, general surgery, and obstetrics/ gynecology wards. Patients admitted in hospital and fitting in the definition of HAI were included in the study. CDC's CLABSI case definitions[2,11]were used to label a case as CLABSI. Only culture proven cases, out of clinically suspected was included in the study for evaluation. Samples (CVC tip and Blood) were collected from suspected CLABSI patients.[2,11]Both active and passive surveillance methods were used for sample collection.Active surveillance was done by visiting various study areas daily along with infection control nurse. Passive surveillance was done by following the positive culture results obtained in microbiology laboratory to retrospective wards in the hospital. The labeled specimens were transported to microbiology laboratory within 30 min of collection. Specimen were inoculated on appropriate culture media including blood agar, MacConkey agar, chocolate agar and incubated for 16-18 hrs at 35-37^{0}C by using standard laboratory techniques.[12,13]Identification of bacteria was based on the colony characteristics of the organism i.e colony morphology, hemolysis on blood agar, changes in the physical appearance of the differential media and enzyme activities of the organisms, Gram staining and biochemical tests.[12,13]Antimicrobial sensitivity was performed on Muller Hinton agar plates by Kirby-Bauer disk diffusion method as per CLSI guidelines.[12-14] Antibiotic discs were procured from HiMedia laboratories, Mumbai, India. Isolates were labeled susceptible, resistant & intermediate on the basis of CLSI disc zone interpretative criterion.[14]All Gram negative isolates were tested for ESBL and MBL production as per CLSI guidelines.[12-14]ESBL positive*Klebsiella pneumonia ATCC* 700603 and ESBLnegative *Escherichia coli ATCC* 25922 were included in the study forquality control of ESBL tests.[14]*Pseudomonas aeruginosa* 27853 was usedas the control for MBL tests.[14]All the media & reagents were procured from Himedia, Mumbai, India.

This study is a part of larger research project titled "Antibiotic pollutants in waters and resistance in rural India-Interventions to improve antibiotic resistance Management (APRIAM)" being conducted in collaboration with KarolinskaInstitutet, Stockholm, Sweden.

RESULT AND DISCUSSION

Presenting data is a part of a prospective cross sectional study in which we assessed prevalence of various HAIs and phenotypic characterization of Gram negative bacterial isolates along with their susceptibility profile. A total of 18536 patients were admitted in the wards under surveillance (surgery wards, obstetrics/ gynecology wards and medical ICU) for HAI during one and half year period (Jan 2012 – June 2013). 6526 patients were excluded from the study because they had a length of hospital stay of less than 48 hours. The remaining 12010 admissions formed study population. The incidence of health care associated infections was (5.14%) and Central Line Associated Blood Stream Infections contributed (0.31%) with 38 culture positive cases. The overall rate of CLABSI was 14.64 per 1000 device days with (0.04) device utilization ratio. (Table-1) Among Gram negative bacterial isolates from CLABSI, *Klebsiella pneumoniae* (40%) was the commonest followed by *E. coli* (26.66%) and *Pseudomonas aeruginosa* (20%). (Table-2) Rest was Gram positive bacterial isolates. Gram negative isolates predominated (79%= 30 out of 38) over Gram positive isolates (21%= 8 out of 38). (Table-2) Among common Gram negative bacterial isolates *Klebsiella pneumoniae* exhibited (100%) resistance to ampicillin, amoxycillin+clavulanic and cefuroximefollowed by (91.66%) to cefotaxime, cefazolin and ceftazidime, (83.33%) to piperacillin and piperacillin + Tazobactum, (75%) to cefepime, gentamicin and ciprofloxacin (58.33%) to amikacin and none of the isolates showed resistance against imipenem. *E. coli* exhibited (100%) resistance to ampicillin, followed by (87.5%) to amoxycillin+clavulanic, piperacillin and cefuroxime, (75%) to cefotaxime, ceftazidime and ciprofloxacin, (50%) to gentamicin and none of the isolates showed resistance against imipenem and amikacin.*Pseudomonas aeruginosa* exhibited (50%) resistance to ciprofloxacin, followed by (33.33%) to piperacillin and piperacillin and piperacillin + Tazobactum, (16.66%) to Amikacin, and none of the isolates showed resistance against imipenem and gentamicin. (Table-3)Among common isolates (37.5%) of *E. coli* and (8.33%) of *Klebsiella pneumoniae* were ESBL producers. None of the isolates of *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were MBL producers. Total (87.5%) isolates of E. *coli,* (91.66%) of *Klebsiella pneumoniae* and (16.66%) of *Pseudomonas aeruginosa* were Multi Drug Resistant (MDR). (Chart-2)

Table-1 Device utilization ratio and incidence and of CLABSI.

Type of HAI	Type of device	Device-days number	Patients days number	Device utilization ratio	Culture positive DAI number	Rate per 1000 device-days
CLABSI*	CVC*	2594	61491	0.04	38	14.64

*** central venous catheter**

Table-2 Gram negative bacterial isolates from CLABSI (n=30)

Microorganisms	Number	(%)
Klebsiella pneumoniae	12	40
E. coli	08	26.66

Pseudomonas aeruginosa	06	20
Citrobacterfreundii	01	3.33
Citrobacterkoseri	01	3.33
Enterobacter aerogenes	01	3.33
Klebsiellaoxytoca	01	3.33

Table-3 Resistance pattern of common Gram negative bacterial isolates from CLABSI (% resistance)

Antibiotics	***Klebsiella pneumoniae*** **(n=12)**	***E. coli*** **(n=08)**	***Pseudomonas aeruginosa*** **(n=06)**
Ampicillin	12 (100)	08 (100)	ND
Amoxycillin + Clavulanic acid	12 (100)	07 (87.5)	ND
Piperacillin	10 (83.33)	07 (87.5)	02 (33.33)
Piperacillin + Tazobactum	10 (83.33)	04 (50)	02 (33.33)
Cefazolin	11 (91.66)	07 (87.5)	ND
Cefepime	09 (75)	06 (75)	01 (16.66)
Cefotaxime	11 (91.66)	06 (75)	ND
Cefoxitin	08 (66.66)	05 (62.5)	01 (16.66)
Ceftazidime	11 (91.66)	06 (75)	00 (00)
Cefoparazone	ND	ND	01 (16.66)
Cefuroxime	12 (100)	07 (87.5)	ND
Imipenem	00 (00)	00 (00)	00 (00)
Amikacin	07 (58.33)	00 (00)	01 (16.66)
Gentamicin	09 (75)	04 (50)	00 (00)
Ciprofloxacin	09 (75)	06 (75)	03 (50)
Trimethoprim/ Sulfamethoxazole	08 (66.66)	01 (12.5)	ND

Chart-1 Resistance pattern of common Gram negative bacterial isolates from CLABSI (%) resistance

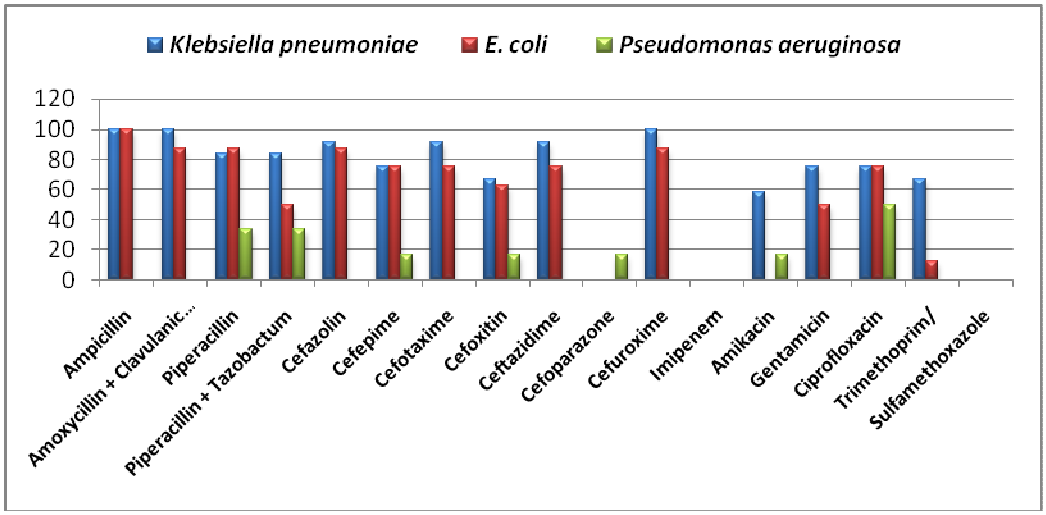
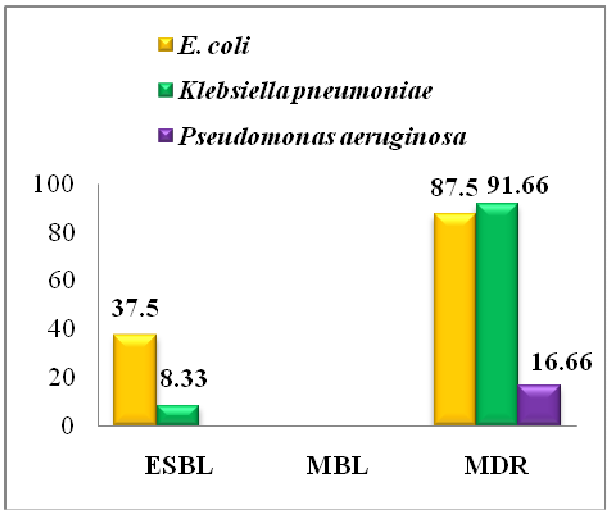


Chart-2 ESBL,MBL and MDR among common Gram negative bacterial isolates of CLABSI.



OBSERVATIONS

Nosocomial infections acquired due to insertion of devices are serious cause of concern to the health care facility.[15,16]Data obtained from surveillance of device associated infections are of great help to the clinicians in treatment of such infections and for health care settings to develop effective infection control policies. To the best of my knowledge there has been very little information available on health care setting DAI rate in the literature from India.[16]In order to determine CLABSI, a total of 2594 central venous catheter days, in 287 cases of central venous catheterization in various wards and ICUs were studied in present study. CLABSI was confirmed microbiologically in 38 cases out of a total 98 clinically suspected cases.The study showed a lower use of CVC (0.04 vs 0.50) compared with the device utilization reported by the US in the NNISS network[15] and (0.70) by INICC.[17]The percentage distribution of CLABSI among HAI was (6.15%) which was lower than

INICC overall CLABSI (30%)[17] and (61.3%) by A. Mehta *et al.*[7]The overall rate of CLABSI was 0.31% or 14.64 per 1000 device days. This is quite high in comparison to other Indian, US and Turkish studies,[7,15,18]while the rate is comparable with the overall INICC rate of 12.5 per 1000 device days.[17]Antimicrobial resistance is a worldwide problem that needs urgent attention.[19] Though this is a worldwide scenario , but situation in India is vulnerable because of injudicious use and over the counter availability of antimicrobials.[20]Insertion of among chronically ill patients have become necessary tool for the successful treatment.Though the insertion of indwelling devices have their own disadvantages like an associated risk of morbidity and mortality.[8]In the present study, *K. pneumoniae* (40%) was the most common isolate followed by *E. coli* (26.66%) and *Pseudomonas aeruginosa* (20%). Results are not consistent with the findings of other observers.[8,21-23] All the isolates of *K. pueumoniae*were resistant to ampicillin and amoxicillin/clavulanic acid. Amikacin was resistant to (58.33%) isolates and was lowest among all commonly used antibiotics. Similar resistance reported by Datta*et al* (59%).[24]Fortunately no resistance was observed for carbapenems in isolates of *K. pneumoniae*. This signifies the prudent use of this group in future in study setup.Hundered percent isolates of *E. coli* were resistant to ampicillin. While no resistance was found to carbapenems and amikacin in this study. These results are not in match with Datta et al, who showed (10% & 57%) resistance to carbapenems and amikacin respectively.[24] Among Pseudomonas aeruginosa no resistance was found for ceftazidime, carbapenems and gentamicin. Among common isolates (37.5%) of *E. coli* and (8.33%) of *Klebsiella pneumoniae* were ESBL producers. None of the isolates of *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were MBL producers. Total (87.5%) isolates of E. *coli,* (91.66%) of *Klebsiella pneumoniae* and (16.66%) of *Pseudomonas aeruginosa* were Multi Drug Resistant (MDR). These findings are in accordance with some and are not in accordance with other researcher's findings.[25-30] Different hospital setup, use of antibiotics and infection control policies among different institutes may be the reasons for varied results.

CONCLUSION

Data from present study indicates that resistance for most of the classes of antibiotics has increased up to the alarming level. Timely action and continuous surveillance of CLABSI and susceptibility pattern of isolates is a need of time for the better management of CLABSI cases and for the development of better infection control policies.

REFERENCE

[1] Rello J, Ochagavia A, Sabanes E, Roque M, Mariscal D, Reynaga E, *et al*. Evaluation of outcome of intravenous catheter-related infections in critically ill patients. *Am J RespirCrit Care Med* 2000; 162 (3 Pt 1):1027-30.

[2] Pittet, D. *et al*. Infection control as a major World Health Organization priority for developing countries. *J Hosp Infect*. **2008**;68:285-92.

[3] Edwards JR, Peterson KD, Andrus ML, Tolson JS, Goulding JS, Dudeck MA, *et al*. National Healthcare Safety Network (NHSN) Report, data summary for 2006, issued June 2007. *Am J Infect Control* **2007**;35:290-301.

[4] Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, *et al*; EPIC International Advisory Committee. The prevalence of nosocomial infection in intensive care units in Europe: results of the European Prevalence of Infection in Intensive Care (EPIC) Study. *JAMA* **1995**;274:639-44.

[5] Safdar N, Crnich CJ, Maki DG. Nosocomial infections in the intensive care unit associated with invasive medical devices. *Curr Infect Dis Rep* **2001**;3:487-95.

[6] Mehta A, Rosenthal VD, Mehta Y, Chakravarthy M, Todi SK, Sen N, *et al*. Device-associated nosocomial infection rates in intensive care units of seven Indian cities. Findings of the International Nosocomial Infection Control Consortium (INICC). *J Hosp Infect* **2007**;67:168-74.

[7] Kaur R, Mathai AS, Abraham J. Mechanical and infectious complications of central venous catheterisations in a tertiary-level intensive care unit in northern India. *Indian JAnaesthesia* **2012**;56:376-81.

[8] Patil HV, Patil VC, Ramteerthkar MN, Kulkarni RD. Central venous catheter-related bloodstream infections in the intensive care unit. *Indian J Crit Care Med* **2011**;15:213-23.

[9] Beekmann SE, Henderson DK. Infections caused by percutaneous intravascular devices. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Disease. Vol 1. 6th ed. Philadelphia, PA: Elsevier; **2005**:3347-3361.

[10] World Health Organization. *World alliance for patient safety:forward*programme 2006-2007. (WHO press: Geneva, Switzerland, **2006**)

[11] deLissovoy, G. *et al*. Surgical site infection: incidence and impact on hospital utilization and treatment costs. *Am J Infect Control.* **2009**;37:387-97.

[12] Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. *Am J Infect Control* **1988**;16:128–40.

[13] Noakes, T. D., J. Borresen, T. Hew-Butler, M. I. Lambert, and E. Jordaan. Semmelweis and the aetiology of puerperal sepsis 160 years on: anhistorical review. *Epidemiol Infect*. **2008**;136:1–9.

[14] Demain, A. L., and R. P. Elander. **1999**. The beta-lactam antibiotics: past, present, and future. Antonie Van Leeuwenhoek 75:5–19.

[15] National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *Am. J. Infect. Control*. **2004**;32:470–85.

[16] Singh S, Pandya Y, Patel R, Paliwal M, Wilson A, Trivedi S. Surveillance of device-associated infections at a teaching hospital in rural Gujrat- India. *Indian Journal of Medical Microbiology*. **2010**;28:342-7.

[17] Rosenthal VD, Maki DG, Salomao R, et al. Device-associated nosocomial infections in 55 intensive care units of 8 developing countries. *Ann Intern Med.* **2006**;145:582-91.

[18] Inan D, Saba R, Yalcin An, Yilmaz M, Ongut G, Ramazanoglu A, *et al.* Device-associated nosocomial infection rates in Turkish medical-surgical intensive care units. *Infect Control HospEpidemiol*. **2006**;27:343-8.

[19] Wattal C. "Antibiotic policy": why and for whom. *JIMSA*.**2004**;17:170-73.

[20] Wattal C, Goel N, Oberoi Jk, Raveendran R, Datta S, Prasad KJ. Surveillance of Multidrug Resistant Organisms in a Tertiary Care Hospital in Delhi, India. *JAPI*.**2010**;58:32-36.

[21] Moonens F, el Alami S, Van Gossum A, Struelens MJ, Serruys E. Usefulness of gram staining

of blood collected from total parenteral nutrition catheter for rapid diagnosis of catheter related sepsis. *J ClinMicrobiol*. **1994**;32:1578–9.

[22] Velasco E, Thuler LC, Martins CA, Nucci M, Dias LM, Gonçalves VM, et al. Epidemiology of bloodstream infections at a cancer center. *Sao Paulo Med J*. **2000**;118:131–8.

[23] Schwaber MJ, Krasner CN, Gold HS, Venkataraman L, Avigan DE, Karchmer AW, et al. Detection of Staphylococcus aureus in peripheral blood stem cell cultures after sterilization of standard blood cultures. *J ClinApher*. **2003**;1:37–9.

[24] Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad KJ. A ten year analysis of multi-drug resistant blood stream infections caused by *Escherichia coli* &*Klebsiella pneumoniae* in a tertiary care hospital. *Indian J Med Res*. **2012**;135: 907-12.

[25] Deepti, Sanjeev Sinha, Surendra K. Sharma, Praveen Aggarwal, Ashutosh Biswas, Seema Sood, et al. Central Venous Catheter Related Bloodstream Infections in Medical Intensive Care Unit Patients in a Tertiary Referral Centre. *The Indian Journal of Chest Diseases & Allied Sciences* **2014**;56:85-91.

[26] Lin K-Y, et al., Central line-associated bloodstream infections among critically-ill patients in the era of bundle care, Journal of Microbiology, Immunology and Infection (**2015**), http://dx.doi.org/10.1016/j.jmii.2015.07.001

[27] Carlos M. Luna, Eduardo Rodriguez-Noriega, Luis Bavestrello, andManuelGuzmán-Blanco. Gram-Negative Infections in Adult Intensive Care Units of Latin America and the Caribbean. *Critical Care Research and Practice* **2014**:1-12.

[28] Miguel Marcos. et al. Changing epidemiology of central venous catheter-related bloodstream infections: increasing prevalence of Gram-negative pathogens. *J AntimicrobChemother* **2011**; 66: 2119–2125.

[29] RupamGahlot, Chaitanya Nigam, Vikas Kumar, Munesh Gupta.Catheter Related Blood Stream Infections in ICU: A study from North India. *Int J Infect Control* **2013**, v9:i2.

[30] Deron C. Burton, et al. Methicillin-Resistant *Staphylococcus aureus* Central Line–Associated BloodstreamInfectionsin US Intensive Care Units, 1997-2007. *JAMA* :**2009**:301;727-736.