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Isolation of aerobic bacteria in central venous catheter associated bloodstream infection in intensive care units of tertiary care hospital (P.B.M and A & G of Hospitals, Bikaner) & determination of antimicrobial susceptibility pattern of isolates

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Abstract
Aims and Objectives: Present study was conducted to find out Incidence rate, Aerobic bacterial spectrum and their Antimicrobial susceptibility pattern from Catheter related blood steam infection over 1 year study.
Material and Methods: Total 75 CVCs and simultaneously withdrawn blood from peripheral site for blood culture were obtained from patents admitted to ICUs, wards and Dialysis units. Catheter Tip was processed by Maki's rolled plate method (Semi-quantitative Culture) [4] and simultaneously obtaining blood culture from peripheral site.
Result: In 75 patients with total catheter days 890, total CLABSI cases were 22.66% (17/75) and the rate of CLABSI was found to be 19.10 per 1000 catheter days. Most common organism causing Catheter related blood stream infection in our study was Coagulase-Positive Staphylococcus aureus (20.58%). MRSA accounted for 11.76% and 8.82% were extended spectrum beta-lactamase (ESBL) producing Klebseilla.
Conclusion: Since central venous catheters are increasingly being used in critical care, they are important source of infection and bacteremia. Duration of catheter is important risk factor for catheter colonization and CLABSI. In our study duration of catheter more than seven days was associated with higher colonization rate. Hence regular surveillance for infection associated with them is essential.

Keywords: central line associated bloodstream infection, central venous catheter, semi quantitative culture

Introduction
Use of vascular catheters is common in both inpatient and outpatient care. CVCs plays an integral role in modern Healthcare, there use however is associated with a risk of bloodstream infections caused by microorganism colonizing the external surface of device or the fluid pathway when the device is inserted or in course of its use [1].CVCs are most frequent cause of Healthcare associated bloodstream infections [2]
Antimicrobial resistance is a problem with all common pathogens that cause CLABSI particularly in ICU's, MRSA account more than 50% of all staphylococcus aureus isolates obtained in ICU's [3].
Present study is undertaken to diagnose Catheter related blood stream infection by using Semi quantitative catheter tip culture (Maki's Roll Plate method) [4] and simultaneously obtaining blood culture from peripheral site.

Material & Methods
Type of study
The present study has been carried out in department of Microbiology & Immunology, Sardar Patel Medical College, Bikaner (Rajasthan) from 1st August 2016 to 31st July 2017.

Sample size
Total 75 CVC's tips and simultaneously peripheral blood is collected for culture from patients admitted in ICU's/ wards/ Dialysis unit.

Inclusion criteria
Patient with following criteria were included for study:
1. Age more than 18 years
2. Central venous line in place inserted first time in Emergency wards, ICU's or Dialysis unit.
3. Placement of CVC line > 48 hours.

Sample collection and processing
CVC are removed under strict Aseptic precaution. The distal end was held over a sterile screw capped container and terminal 4-5cm segment of catheter (tip) was cut with sterile scissor and were collected and transport to lab as soon as possible within 15 min [4, 5, 6].

Catheter tip processing
Catheter tip were processed by using Extra luminal Maki's Roll over Plate method (Semi-quantitative culture technique) [4]. Using sterile forceps, distal segment (catheter tip) was removed from sterile container and laid on Blood agar plate and rolled back and forth across the entire surface of blood agar plate using sterile forceps, exerting slight downwards pressure. BA plates were then incubated for 48-72 hours at 37°C and were examined daily and colonies were counted as soon as growth was observed. The results were expressed as CFU (figure 1, 2).

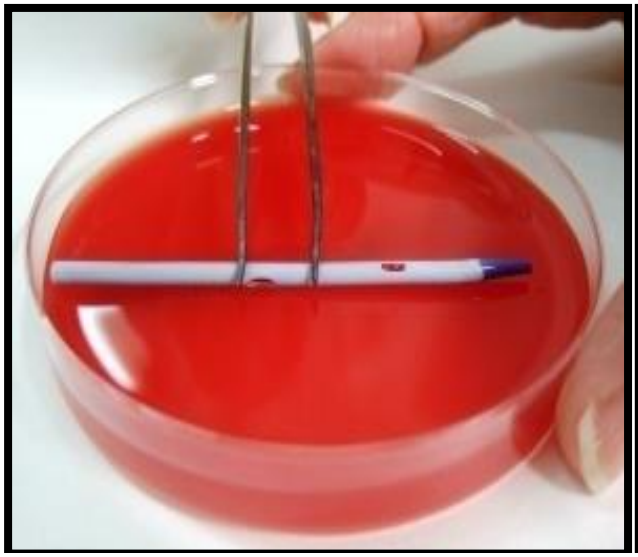


Fig 1: Catheter Tip

Fig 2: Maki's Roll Plate

Interpretations
Blood Agar plates were examined at 24hrs, 48hrs and 72 hrs. Significant growth was defined as 15 colony forming units (CFU) or more by Maki's Roll Plate method Antimicrobial sensitivity were performed in all potential pathogens with growth> 15 CFU (figure 3).Mixed culture with > 15 CFU were evaluated by case by case basis.
Isolation of < 15CFU is associated with catheter contamination with normal skin flora. (Figure 4).

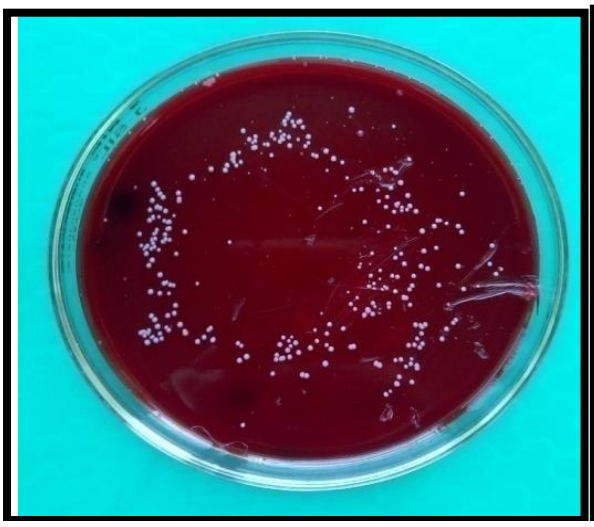


Fig 3: Growth >15 CFU

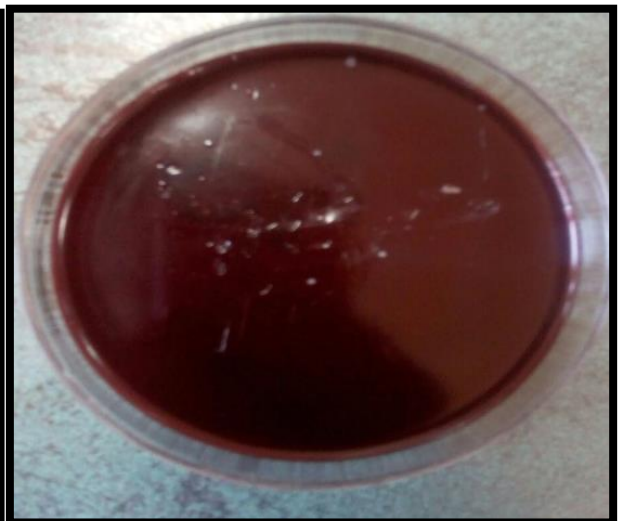


Fig 4: Growth <15 CFU

Blood Culture [6, 12, 13]
Manual conventional blood culture was used for simultaneous peripheral blood culture for the patients.

Antimicrobial drug sensitivity of isolates was determined using standard technique, Kirby Bauer disc diffusion method as per CLSI guidelines.

Incidence Rate Calculation

The rate of CLABSI in a patient population is calculated as per below formula –

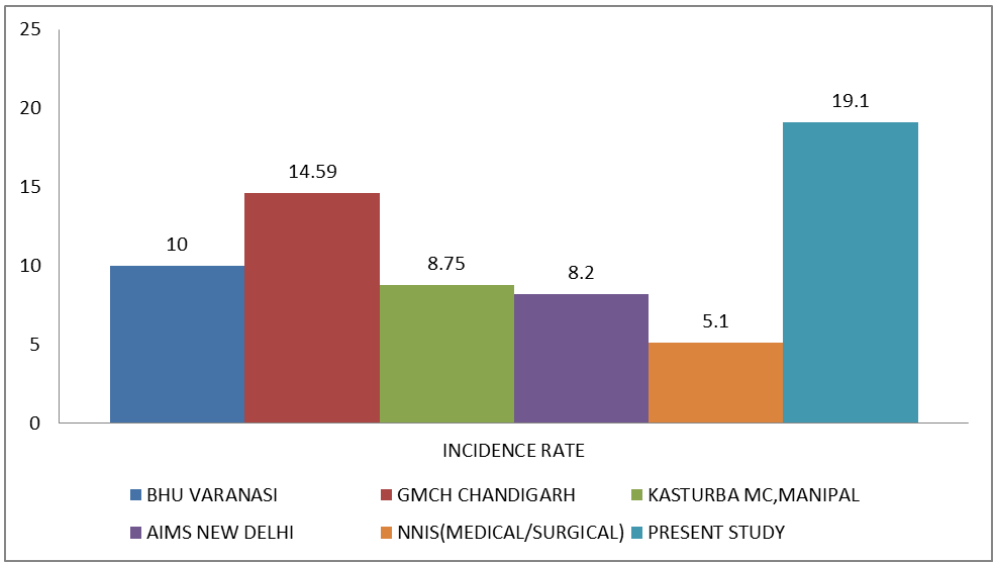
$$\text{INCIDENCE RATE (per 1000 catheter days)} = \frac{\text{No.of CLABSI Cases}}{\text{No. of CVC days}} \times 1000$$

Central line days, not patient days are used as denominator, as only patient with a central line are at risk of developing a CLABSI [10].

Result

Table 1: Comparison of Incidence rate per 1000 catheter days

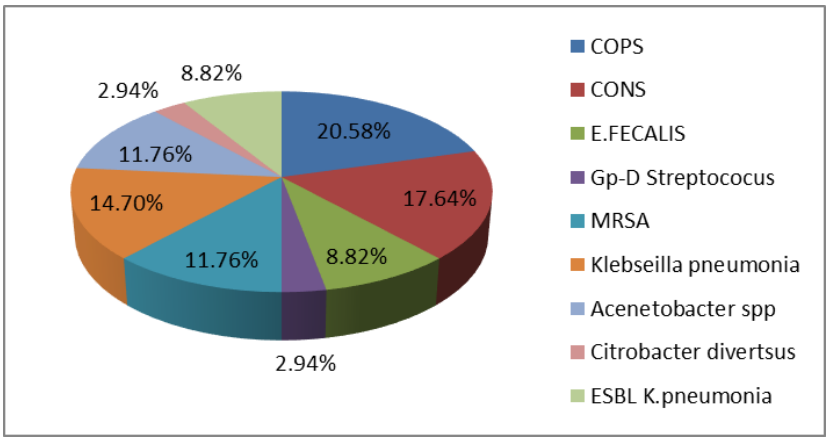
Institution	Incidence Rate (per 1000 CL Days
1. BHU Varanasi, IMS	10
2. GMCH,Chandighar	14.59
3. Kasturba medical college,Manipal	8.75
4. AIIMS,New Delhi	8.2
5. NNIS(Medical/Surgical)	5.1
6. Present study	19.1



Graph 1: Comparison of Incidence rate per 1000 catheter days

Table 2: Aerobic bacterial Isolates in CLABSI cases

1	COPS	7 (20.58%)
2	CONS	6 (17.64%)
3	E.FECALIS	3 (8.82%)
4	Gp-D Streptococcus	1 (2.94%)
5	MRSA	4 (11.76%)
6	Klebsiella pneumonia	5 (14.70%)
7	Acenetobacter spp	4 (11.76%)
8	Citrobacter diversus	1 (2.94%)
9	ESBL Klebsiella	3 (8.82%)



Graph 2: Aerobic bacterial Isolates in CLABSI cases

Table 3: Antibiotic Sensitivity Pattern of Aerobic Gram Positive Bacterial Isolates (CLABSI cases)

Antibiotics	Cops (N=7)	Cons (N=6)	E. Fecalis (N=3)	Group-D Streptococci (N=1)	Mrsa (N=4)
Amikacin	6 (85.71%)	3 (50%)	2(66.66%)	NIL	3(75%)
Azithromycin	2(28.57%)	NIL	NIL	NIL	NIL
Ceftriaxone	6 (85.71%)	6 (100%)	1(33.33%)	1 (100%)	1(25%)
Cotrimox	3 (42.85%)	5 (83.33%)	NIL	NIL	2(50%)
Ciprofloxacin	2(28.57%)	5 (83.33%)	1(33.33%)	1(100%)	3(75%)
Clindamycin	2 (28.57%)	2 (33.33%)	1(33.33%)	1(100%)	1(25%)
Oxacillin	7 (100%)	5 (83.33%)	NIL	NIL	NIL
Vancomycin	7(100%)	6 (100%)	3(100%)	1(100%)	4(100%)

(The percentage within parenthesis expresses the number of strain sensitive to particular antibiotic of total organism isolated).

Table 4: Antibiotic sensitivity pattern of Gram negative isolates (CLABSI cases)

Antibiotics	K.pneumonia (n=5)	Acenetobacter (n=4)	C.diversus (n=1)	ESBL(KLEB) (n=3)
Ampicillin	4(80%)	2(50%)	NIL	1(33.33%)
Amoxyclav	4(80%)	3 (75%)	1(100%)	2(66.66%)
Ceftrioxone	4(80%)	1(25%)	NIL	NIL
Cotrimox	1(20%)	NIL	NIL	NIL
Ciprofloxacin	4(80%)	1(25%)	NIL	NIL
Cefoperazone	2(40%)	NIL	NIL	1(33.33%)
Gentamycin	2(40%)	1(25%)	NIL	2(66.66%)
Meropenem	5(100%)	4(100%)	1(100%)	3(100%)

(The percentage within parenthesis expresses the number of strain sensitive to particular antibiotic of total organism isolated)

Table 5: Catheter Duration versus Catheter colonization.

Catheter Days	Catheter Culture >15 Cfu (N17)
<7 DAYS	Nil
7-14 DAYS	2(11.76%)
14-21 DAYS	9 (52.94%)
>21 DAYS	6(35.29%)

Clabsi rate

Among 75 patients with total catheter days 890, only 17(22.16%) were found to be of CLABSI cases. The rate of CLABSI was 19.10 per 1000 catheter days.

Aerobic bacteriological profile

In our study of total aerobic bacterial isolates among CLABSI cases, Gram positive cocci were 61.76% and Gram negative bacilli were 38.23%.Most common GPC were COPS 20.58% f/b CONS 17.64%.and most common GNB were Klebsiella pneumonia14.70% f/b Acenetobacter species 11.76).MRSA were 11.76% and ESBL producing Klebsiella pneumonia were 8.82% among total aerobic bacterial isolates among CLABSI cases. (Table 2, Graph2)

Antibiotics sensitivity pattern among CLABSI isolates gram positive bacteria's

Of total 21 GPC including COPS (7), CONS (6), Enterococcus fecalis (3), Group-D Streptococcus (1) and MRSA (4), all were 100% sensitive to Vancomycin. (Table 3).

Gram negative bacteria

Total 13 GNB including Klebsiella pneumonia (5), Acenetobacter spp (4), Citrobacter diversus (1) and ESBL producing Klebsiella pneumonia (3) all were 100% Sensitive to Meropenem. (Table 4).

Duration of catheter

In our study mean duration of catheter were 18.47 days among total 17 CLABSI cases. Duration of catheter more than 7 days was associated with higher catheter colonization. (Table 5).

Discussion

The rate of CLABSI was found to be 19.10 per 1000 catheter days. which is much higher when compared to the study conducted by National Nosocomial Infection Surveillance System (NNIS) from Jan 1992 to June 2004 shows the median rate of CRBSI in ICUs of all type ranged from 1.8 to 5.2 per1000 catheter [10, 11] Incidence rate in our study was also higher when compared to various study conducted in India [10-12, 15, 17, 18] (Table 1, Graph 1.) In present study,higher rates could be attribute to poor nurse patient ratio, compromised infection control practices, longer duration of catheterization and critically ill patients with preexisting systemic disease like sepsis pneumonia P.U.O.

In our study of total aerobic bacterial isolates among CLABSI cases, Gram positive cocci were 61.76% and Gram negative bacilli were 38.23%.Most common GPC were COPS 20.58% f/b CONS 17.64%.and most common GNB were Klebseilla pneumonia14.70% f/b Acenetobacter species 11.76). In the study of Ramanathan Parameswaran *et al.,* (2011) [12] 64% of the pathogens causing CRBSI were Gram positive and 36% were Gram negative. The commonest pathogen causing CRBSI was S.aureus 40%, *Pseudomonas aeruginosa* 16%, coagulase negative staphylococci 8%, *Klebsiella pneumonia* 8% and *Acinetobacter baumanii* 4%.

Over the years there has been a change in the etiology and sensitivity patterns of CLABSIs with increase incidence of Gram negative bacteria, coagulase negative Staphylococci, followed by Enterococci [16]

In our study of the total aerobic bacterial isolates among CLABSI cases, MRSA were 11.76% and ESBL producing Klebseilla pneumonia were 8.82%. All gram positive bacteria causing CLABSI were 100% sensitive to vancomycin and all Gram negative bacilli were 100% sensitive to meropenem. (Table 3, 4). In a study of Ramanathan Parameswaran *et al* (2011) [12] the antibiotic

sensitivity profiles showed that 6.3% were ESBL producing organisms, 30.2% were multidrug resistant (MDR) and among the Staphylococci isolated, 31% were MRSA and 69% were methicillin sensitive (MSSA). In patients with local catheter infections, both the MRSA and methicillin resistant coagulase negative Staphylococci isolated were 100% sensitive to Vancomycin, Tecoplanin, and Linezolid. *Pseudomonas aeruginosa* isolates were sensitive to Cefoperazone - sulbactum, Piperacillin - Tazobactum, Ticarcillin - Clavulinic acid (85.7% for each antibiotic) and Meropenem (78.6%), the Escherichia coli were sensitive to Cefuroxime and Meropenem (88.9% for each antibiotic) and the *Klebsiella pneumoniae* were sensitive to Amikacin (12.5%) and Meropenem (50%). Only one strain of *Acinetobacter baumannii* isolated from a patient with CRBSI was resistant to all routine and reserved drugs.In a study of M Kaur *et al* (2015) [18] multidrug resistance was observed in almost all the isolates responsible for CVC-BSI and catheter colonization. Amongst CVC-BSI isolates, MRSA (87.50%), VRE (25%), MBL (33.33% of Acinetobacter Species) and ESBL (100% of *K. pneumoniae*) were seen. *Pseudomonas aeruginosa* was resistant to amikacin and gentamicin [17].

Conclusion

Present study is undertaken to diagnose Catheter related blood stream infection by using Semi- quantitative catheter tip culture (Maki's Roll Plate method) [4] and simultaneously obtaining blood culture from peripheral site.(conventional blood culture). The rate of CLABSI was found to be 19.10 per 1000 catheter days. Duration of catheter is important risk factor for catheter colonization and CLABSI. In our study duration of catheter more than seven days was associated with higher colonization rate and CLABSI. Since central venous catheters are increasingly being used in critical care, regular surveillance for infection associated with them is essential.

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