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Microbiological Profile and Antimicrobial Susceptibility Testing of Isolates from Central Line Catheters in Patients from Medical Intensive Care Unit of Tertiary Care Hospital - A Recent Changing Trend

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A B S T R A C T

Blood stream infections (BSI) in patients admitted to Intensive care unit (ICU) has a high fatality rate as these patients also have associated comorbid conditions. One of the life saving invasive procedures includes the central line which introduces infection and makes them more vulnerable to BSI. Laboratory processing of Central line to detect such infections becomes mainstay to differentiate infection from colonization. Infection with multi drug resistant organisms (MDRs)is on rise and treating such highly resistant organism faces a great challenge to the treating clinician. To assess the microorganisms causing such infection related to central line and perform their Antimicrobial susceptibility testing. Processing of central line catheters with relevant clinical samples was done under proper aseptic techniques. Among study population, 47(21.36%) patients developed central line related local infection and 7 (3.18%) patients developed central line associated blood stream infection (CLABSI). Gram negative isolates (71.42%), Gram positive isolates (14.28%), and *Candida albicans* (14.28%) were common isolates from CLABSI. Gram negative isolates were predominant in causing local and systemic infections related to catheter.

Introduction

Central line insertion is an important procedure in ICU (Intensive care unit) settings. Although it is a life saving technique morbidity is increased by itself if proper precautions are not followed during insertion and removal. Infection is one of the major complications amongst the others as it tends to increase the mortality in these Patients (Yardena *et al*., 1997). Antimicrobial susceptibility testing is a major issue in these patients with central line as more often resistant strains are isolated and difficult to treat. Central line related Local (CRLI) and Central line associated bloodstream infections (CLABSI) are the two swords in infection related to

central line. Thus isolation of the microbe causing such complication and its susceptibility testing faces a major challenge to a microbiologist.

Many techniques have been followed in practice to detect central line associated blood stream infections, but the noteworthy of all stands the roll technique method which is a semiquantitative method for detecting this infection. Its major advantage being not only the detection of microbe but also a standard cut off for the colony forming units (CFU) which helps the consultant to relate the growth with infection (Maki *et al*., 1977). So the purpose of our study was to detect the organism causing CRLI and CLABSI and to perform their antimicrobial susceptibility testing.

Materials and Methods

A prospective study was undertaken from in the department of microbiology in collaboration with Medical Intensive Care Unit (MICU) at Lokamanya tilak medical college and hospital, Mumbai. Two hundred and twenty consecutive adult patients on central line were the study populates. Ethical committee clearance was taken.Patients who developed local and systemic signs of infection after 48hrs constituted the inclusion criteria of the study. Patients who developed infection after 48hrs but due to cause other than central line were excluded. To exclude such patients samples like Endotracheal secretions, blood, urine, pus and lastly central line were collected and transported under aseptic technique and processed in laboratory. Patient's clinical details including all risk factors, complete hemogram, serum electrolyte levels were also recorded. Two procedures were followed to detect the organism. Firstly Semiquantitative Maki's roll over technique was followed by Quantitative flush technique method (Linares *et al*., 1985). Antimicrobial susceptibility testing of isolated pathogens to clinically relevant antimicrobials was performed by Kirby Bauer diffusion methods, according to the guidelines published by the Clinical and Laboratory Standards Institute (CLSI) (Clinical Laboratory and Standards Institute, 2010).

Central line related local infections (crli) was diagnosed as (Leonardo *et al*., 2005)

1. Any sign of local infection (induration, erythema, heat, pain, purulent drainage) and
2. Catheter tip colonization was defined as "Significant growth of a microorganism by

a) >15 colony-forming units from the catheter tip by semiquantitative method or

b) $>10^3$ by quantitative culture."

Central line associated blood stream infections (CLABSI) was diagnosed as (CDC/NHSN)

Recognized pathogen isolated from blood culture and pathogen not related to infection from another site (other than site of an intravascular device i.e. it should not have been isolated from urinary tract / respiratory tract / wound, etc)

OR

One of the following –

1. fever (>38 C)
2. chills
3. hypotension

AND any of the following:

(a) Common skin contaminant isolated from two blood cultures drawn on separate occasions, and organism is not related to infection at another site.

(b) Common skin contaminant isolated from blood culture from patient with intravascular access device and physician institutes appropriate antimicrobial therapy.

(c) Positive antigen test on blood or organism is not related to infection at another site.

Results and Discussion

During the study period a total of 220 consecutive adult patients with central venous catheter were analysed. Of these 47(21.36%) patients developed Catheter related local infection (CRLI) and seven 7(3.18%) patients developed Central line associated blood stream infections (CLABSI). Distribution of organisms causing local and systemic catheter infections are depicted in tables 1 and 2 respectively.

MSSA (21.27%) was the most common isolate causing local infection, followed by *Klebsiella pneumoniae* (19.14%) and *Acinetobacter* spp. (17.02%). Isolates from CLABSI included 3 (42.85%) were *Acinetobacter spp*, 2 (28.57%) *Klebsiella pneumonia* and 1(14.28%) MSSAand 1(14.28%) *Candida albicans.*

Percent distribution of organisms causing CLABSI is shown in Fig 1 which depicts predominance of gram negative isolates.

Antimicrobial susceptibility

From CRLI

All MSSA isolates showed 100% sensitivity to ciprofloxacin (Cf) and cefuroxime (CXM), while 0% were sensitive to penicillin G (PG). None of the isolates showed Inducible Clindamycin resistance.

All MRSA (n=4)isolates showed 100% susceptibility to vancomycin, linezolid and netilimicin. While only one isolate was susceptible to gentamicin(25%). None of the isolates showed Inducible Clindamycin resistance.

All CONS (n=3)were sensitive to ciprofloxacin and cefoxitin (100% each).Only 66.7% susceptibility was seen to cefuroxime.0% sensitivity was seen to penicillin G, gentamicin and cotrimoxazole.

Two *Enterococcus* spp isolated showed 100% sensitivity to vancomycin and linezolid. 0% sensitivity was seen to Penicillin G and gentamicin. Two isolates were negative for high level aminoglycoside resistance

Of nine isolates of *Klebsiella pneumonia*, six were sensitive to amikacin (66.7%). There was 0% sensitivity to piperacillin, ciprofloxacin, cefotaxime, amoxiclav and gentamicin. Of three isolates which were resistant to primary line of antibiotics tested, all were sensitive to imipenem (100%), one was sensitive for netilimicin (33.3%) none(0%) to cefepime, ceftriaxone-sulbactum, cefaperazone–sulbactum.

Of eight isolates of *Acinetobacter spp* , four were sensitive to amikacin (50%) and two were sensitive to ciprofloxacin (25%). There was 0% sensitivity to piperacillin, cefotaxime, amoxiclav and gentamicin. Of four isolates which were resistant to primary line of antibiotics tested, all were sensitive to imipenem(100%), two to piperacillin tazobactum (50%), one to netilimicin (25%) and none(0%) to cefepime, ceftriaxone-sulbactum, cefaperazone –sulbactum.

All isolates of *Pseudomonas aeruginosa* showed sensitivity to amikacin (100%). While only 85.7% were sensitive to piperacillin, 71.42% to ciprofloxacin, 57.14% to ceftazidime and only 42.85% to gentamicin respectively.100% sensitivity was seen for amikacin, piperacillin and ciprofloxacin of single isolte of *Citrobacter spp*. 0% sensitivity was seen to cefotaxime, amoxiclav and gentamicin.

From CLABSI

Of three *Acinetobacter spp* isolated, 66.6% were sensitive seen to amikacin and 33% sensitive to ciprofloxacin and piperacillin each. 0% sensitivity was observed for cefotaxime, amoxiclav and gentamicin. Primary line resistant *Acinetobacter sp* was tested for higher antibiotic drug susceptibility and it was found to be sensitive to imipenem (100%), 0% sensitive to netilimicin, piperacillin-tazobactum, cefepime, ceftriaxone-sulbactum and cefaperazone-sulbactum.

Of two *Klebsiella pneumoniae* isolated, 50% were sensitive to amikacin and 0% sensitive to piperacillin, ciprofloxacin, cefotaxime, amoxiclav and gentamicin. Primary line resistant *Klebsiella pneumoniae* was tested for higher antibiotic drug susceptibility and it was found to be sensitive to imipenem (100%), 0% sensitive to netilimicin, piperacillin-tazobactum, ceftriaxone-sulbactum, cefaperazone-sulbactum and cefepime as shown in Fig 2.

MSSA isolate showed 100% sensitivity to ciprofloxacin and cefuroxime, while it showed resistance to penicillin G, cotrimoxazole and gentamicin. The isolate did not show Inducible Clindamycin resistance.

There were 47 patients who developed local infection due to central venous catheter. Semiquantitative Maki's roll over technique was positive in all 47 patients but quantitative flush technique was positive in 43 patients. Growth was not seen in 4 samples processed by flush technique, but these were positive by roll technique. Slobbe *et al.*, in 2009 demonstrated that the use of the quantitative sonication technique to detect catheter tip colonization in patients with Central Venous Catheters(CVC)s had no surplus value compared with the semiquantitative roll plate method. In another study by Maki *et al.*, in 1997 also observed that semiquantitative technique distinguishes infection (greater than or equal to 15 colonies) from contamination and is more specific in diagnosis of catheter-related septicemia than culture of the catheter in broth.

In the present study, semiquantitative technique (23.5%) turned out to be a better indicator of infection than flush technique (21.5%) similar to the above study.

All 47 patients in the present study had local signs and symptoms of infection like induration, pain, erythema and oozing and their blood cultures, urine, ET secretions and induced sputum were negative.

Central line associated blood stream infections developed in 7 patients who also were blood culture positive and showed growth by both semiquantitative and quantitative flush technique. All patients who developed systemic infection had systemic signs and symptoms of septicemia like fever, hypotension attributable to central line because other samples like urine, ET secretions, sputum and pus showed no growth.

Microbiological spectrum

Infections are known to be one of the important consequences of central venous

catheterization in ICU patients. In the present study of all the central venous catheters processed, 54 specimens were positive by Semiquantitative maki's roll over technique. Out of 54 isolates grown, 47 were isolated from patients who developed CRLI and 7 were isolated from patients who developed CLABSI .

According to a study on intravascular catheter-related infections in an Indian tertiary care hospital in 2011 by Ramanathan *et al.,* the common organisms causing local infections were Coagulase-negative *Staphylococci, Staphylococcus aureus, E. coli, Klebsiella pneumoniae and Acinetobacter spp.*

Of the 47 isolates grown in the present study from patients with CRLI, 27 were Gram negative bacilli (57.44%) and 20 Gram positive cocci (42.56%). Of these 21.27% of these isolates were MSSA, 19.14% *Klebsiella pneumoniae*, 17.02% *Acinetobacter spp*, 14.8% *Pseudomonas aeruginosa*, 8.5% MRSA, 6.3% Coagulase negative *Staphylococcus aureus* (CONS) and 4.2% *Enterococcus spp*.

In the present study, the commonest isolate was MSSA (21.27%) which is comparable to the above study. However only 6.3% CONS were grown in this study. Gram negative organisms were predominant (57.44%) in the present study with the commonest being *Klebsiella pneumonia* (19.14%) and *Acinetobacter spp* (17.02%) which was also comparable with the above study.

Seven organisms were isolated from central venous catheter causing systemic infections in the present study. Gram negative bacilli predominated (71.42%) and 14.28% were MSSA and *Candida albicans* each. Of these 42.85% were *Acinetobacter spp* and 28.57% were *Klebsiella pneumoniae*. The present study correlated with two Indian studies done by Gopalakrishnan *et al.,* and Pawar *et al*., which showed gram negative isolates predominance. However, in a study done by Kevin *et al.,* in 2008 the most commonly identified pathogens were gram-positive organisms; Coagulase-negative *Staphylococcus* species, *Staphylococcus aureus*, and viridans group streptococci, which is in contrast to the present study.

According to a study done by sheik *et al.,* and Gupta *et al., Staphylococcus aureus* and Coagulase negative *Staphylococcus* accounted for majority of CLABSI episodes, the other being Gram negative organisms like *Pseudomonas spp.* and *Escherichia coli* which again doesn't correlate with the present study.

Antimicrobial susceptibility testing

There have been increasing reports of resistance developing in organisms isolated from ICU settings (Ram *et al*., 2010). The antimicrobial susceptibility pattern of these isolates helps the clinician to use appropriate antimicrobial agent and also helps the clinician to deescalate or change the antimicrobial for better management of patients admitted in ICU.

In the present study antibiotic susceptibility of Gram negative isolates varied.

Klebsiella pneumoniae was the most common Gram negative isolate and it showed 66.6% susceptibility to amikacin and 0% susceptibility to other antimicrobials tested. *Pseudomonas aeruginosa* was next common Gram negative isolate which showed 100% sensitivity to amikacin, 85.71% to piperacillin, 71.42% to ciprofloxacin, whereas in *Acinetobacter spp*, only 50% sensitivity was seen to amikacin and 25% sensitivity to ciprofloxacin.

Table.1 Distribution of organisms causing CRLI (n=47)

Gram positive Organisms	Total (%)	Gram negative Organisms	Total (%)
MSSA	10 (21.27%)	*Klebsiella pneumoniae*	9 (19.14%)
MRSA	4 (8.5%)	*Acinetobacter* spp	8 (17.02%)
CONS	3 (6.3%)	*Pseudomonas aeruginosa*	7 (14.8%)
Enterococcus spp	2 (4.2%)	*Enterobacter* sp	1(2.1%)
Streptococcus sp	1 (2.1%)	*Escherichia coli*	1(2.1%)
		Citrobacter sp	1(2.1%)

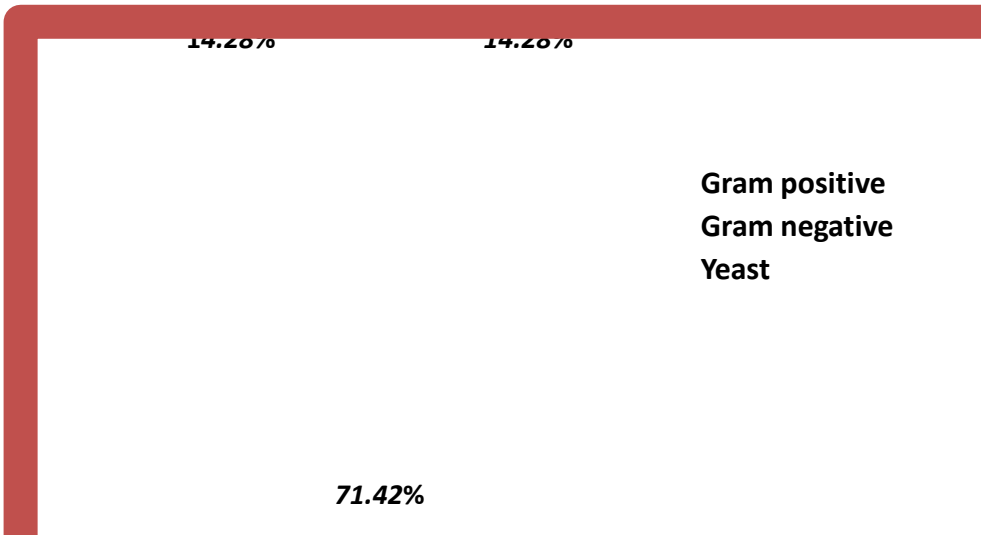
MSSA(Gram positive) was the commonest isolate causing Local infection followed by gram negative isolates

Table.2 Distribution of organisms causing CLABSI(n=7)

Organisms	Total (%)
Acinetobacter spp	3(42.85%)
Klebsiella pneumoniae	2(28.57%)
MSSA	1(14.28%)
Candida albicans	1(14.28%)

Gram negative isolates were commonest causing systemic central line infections.

Fig.1 Distribution of organisms causing CLABSI



Gram negative predominance in CLABSI.

Fig.2


Mueller Hinton agar showing antimicrobial susceptibility test of *Klebsiella pneumoniae*
Resistant to Amikacin, Amoxycillin-clavulanic acid, Cefotaxime and Cefuroxime, Ciprofloxacin
Sensitive to Imipenem; resistant to Piperacillin-tazobactam, Ceftriaxone-sulbactam and Cefepime, Netilimicin

Gram negative isolates were also tested against beta lactam inhibitor combinations, like Piperacillin–tazobactum, Ceftriaxone-sulbactum and Cefaperazone – sulbactum. *Klebsiella pneumoniae* was found to be resistant to all beta lactum inhibitor combinations. *Acinetobacter spp* showed 50 % sensitivity was seen to piperacillin–tazobactum combination and no (0%) sensitivity to ceftriaxone- sulbactum, cefaperazone–sulbactum. Netilimicin sensitivity varied among *Klebsiella pneumonia* (33.3%) and *Acinetobacter spp* (25%).All Gram negative isolates resistant to primary line of antibiotics were tested for sensitivity to carbapenems and all were sensitive to imipenem.

The present study, thus shows variation in the antimicrobial susceptibility pattern when compared to the study done by Ramanathan *et al*., This could be because of the variation in the susceptibility pattern among organisms isolated from MICU which tend to vary from centre to centre and on infection control practices as well (Ram *et al*., 2010).

In the present study seven isolates were grown from blood culture and central venous catheter. *Acinetobacter spp* (42.85%) was the most common isolate followed by *Klebsiella pneumoniae* (28.57%) and MSSA (14.28%).

Gram negative isolates were also tested against beta lactam inhibitor combinations, like Piperacillin–tazobactum, Ceftriaxone-sulbactum and Cefaperazone – sulbactum. *Acinetobacter spp* and *Klebsiella pneumoniae* were found to be resistant to all beta lactum inhibitor combinations. All Gram negative isolates resistant to primary line of antibiotics were tested for sensitivity to carbapenems and all were sensitive to imipenem.

Again there was much variation observed when compared to study done by Ramanathan *et al.,* (2011).

In conclusion, gram negative isolates predominance was seen in both Central line related local infections and central line associated blood stream infections .Although many western literatures show gram positive predominance for blood stream infections in patients with central line recent change has been observed towards gram negative pathogens. But most common isolate causing local infection was *Staphylococcus aureus*.

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