ISSN 2456-4400

Mariappan et al., Int J Med Lab Res 2017, 2(2): 13-21

RESEARCH ARTICLE INTERNATIONAL JOURNAL OF MEDICAL LABORATORY RESEARCH (IJMLR)

BACTERIAL PROFILING OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN BLOOD STREAM INFECTIONS OF SUSPECTED BACTEREMIA PATIENTS FROM KANYAKUMARI DISTRICT, TAMILNADU, INDIA

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Received:27 July, 2017/Revision: 2 August,2017/Accepted:7 August, 2017

ABSTRACT: Antimicrobial resistance pattern of Bloodstream infections in patients with suspected bacteremia from Kanyakumari District, Tamil Nadu, India was surveyed for a period of 6 months and cultured as per the methods employed by CLSI. Antibiotic sensitivity was tested using Kirby-Bauer disc diffusion method. A total of 295 bacteremia suspected patient's blood culture samples were processed, of which 6 bacterial pathogens isolated from 27 positive blood cultures, among which 67% were gram-positive and 33% were gram-negative.The predominant isolate was coagulation negative *Staphylococcus*spp. (CoNS) (37%). The other isolates were *Staphylococcus aureus* (30%), *Escherichia coli* (18%), *Pseudomonas aeruginosa* (7%), *Klebsiella pneumoniae* (4%), and *Enterococcus* spp. (4%). The pathogens coagulase negative *Staphylococcus* spp and *Staphylococcus aureus* were more commonly resistant to Co-trimoxazole (50 to 68%) and Penicillin G (83 to 90%). Bloodstream infections are important causes of morbidity in patients, especially among the age group of 1-20 years. Prescription of proven resistant antibiotics to suspected bacteremic patients' needs utmost attention in the study region.

KEYWORDS: Bloodstream infection- Bacterial Pathogens - Antibiotic resistance and sensitivity

INTRODUCTION:

Bloodstream infections (BSI) (bacteremia) are potentially life-threatening diseases that emerge from the delay in administration of first adequate anti-infectious agent. Rapid microbiological investigations are required for the identification of the correct causative agents and antimicrobial susceptibility testing (AST) for an efficient treatment so as to reduce the spectrum of resistant strains, toxicity and negative impact on beneficial bacteria implications of broad-spectrum antibiotics or a combined therapy needs to be understood carefully. The estimated

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quantity of bacterial pathogens in the blood during BSIs generally ranges from 1 to 10 CFU/ml or 1×10^3 and 1×10^4 CFU/ml (Opota, 2015).

Qualitative and quantitative microbiological results for blood samples may help to establish the clinical significance of bacteremia and can provide clues to determine whether a blood culture sample is a true positive or a false positive (contaminant).For the diagnosis of bacteremia, blood cultures are the most accurate diagnostic test among other techniques (Mitta *et al*., 2009).. The rate of positive culture may be influenced by the type of microorganism involved. Generally, bacteremias are of low-grade with half of the bacteremic patients having less than one organism per mL of blood (Ern Gutschik, 1998).A bacteriological culture of blood is a necessary for identifying infectious agents causing bacteremia and the presence of bacteria may be transient, intermittent or continuous. Blood culture examination aids to determine the source of infection and optimize of antimicrobial therapy. Early identification of pathogens in blood is a crucial step in assuring appropriate therapy, and beginning of an effective antibiotic therapy. This plays a significant role on the outcome of the disease (Garey, 2006). If the patient is already on antimicrobial therapy, recovery of pathogens may be increased by collecting the blood sample immediately before administering the next dose and by inoculating the blood into bottles containing specialized antimicrobial neutralization media. The optimal recovery of pathogens from blood depends on culturing an adequate volume of blood. The collection of a sufficient quantity of blood improves the detection of pathogens present in low quantities. To screen the bacteremia in adults, one blood sampling requires up to 20ml of blood for the culture of both aerobic and anaerobic microbial pathogens. The volume of the blood sample depends on the concentration of organisms and is low in the majority of bacteremias. In infants and children, the concentration of microorganisms during bacteremia is higher than in adults. Therefore, less quantity of blood samples are required for culture. Culturing process has to be repeated for four times with 40 ml to 80 ml of blood in order to detect causative agents in 80% to 96% of bacteremias before administrating antitheraphy (Cockeril, 2004).Cultures should not be taken through indwelling vascular catheters because of the risk of contaminants. The recommended volume of blood to collect should be based on the weight of the patient and an aerobic bottle should be used, unless an anaerobic infection is suspected. (Freedman *et al*., 2004)

Bacteremia is yet to be a major cause of morbidity and mortality among children despite used advanced medicines (Kalantar *et al*., 2008). The organisms responsible for bacteremia vary across geographical boundaries. About 95 % of all bloodstream infections are caused by only 15 different genera of bacteria which mainly include *E. coli*, *Klebsiella* spp., *Staphylococcus aureus*, *Pseudomonas* spp., *Salmonella* spp. and *Acinetobacter* spp. Among them, *Staphylococci* and *E. coli* account for more than 50 % of the infections as their detection and multidrug resistance go beyond limits (Castagnola*et al*., 2005).For the diagnosis of bacteremia, blood cultures are the most accurate diagnostic test among all other techniques (Mitta *et al*., 2009).The rate of positive culture are generally influenced by the type of microorganism involved.

In the light of the above background, this study was aimed to determine their occurrence, identity and evaluate their antimicrobial resistance patterns of bacterial pathogens from the blood supplies of patients with bacteremia from Kanyakumari District of Tamilnadu.

MATERIALS AND METHODS:

Specimen Collection

It has been a routine practice in our authorized medical laboratory, to disinfect skin of patients with 70% alcohol followed by 2% povidone-iodine concentrically for one minute before collecting blood samples (10 ml) by venipuncture method. A total number of 295 blood samples were collected in the laboratory during 1st January 2017 to 30thJune 2017. Out of 295 samples, 38 were collected from adult females, 62 from adult males, 121 from male children and 74 from female children.

Microbiological Processing of Blood Samples

From each sample, 5 ml of blood was inoculated individually into the BACTEC aerobic culture vials and BACTEC aerobic culture vials under aseptic conditions. The inoculated vials were brought to the microbiology laboratory Blood samples were transferred to blood culture media and immediately transported to microbiology laboratory. All blood cultures were then incubated in fluorescent series instrument and growth was monitored. The positive cultures were gram stained to differentiate gram positive and gram negative. The positive culture was then sub cultured on Mac-Conkey agar, Blood agar, Chocolate agar, and anaerobic media (Fluid thioglycollate media) and incubated at 37ºC for 24 to 72 hours. After incubation, the isolated colonies were subjected for identification by conventional biochemical tests as employed by Monica Cheesbrough (2004).

Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was done by disc diffusion method according to the Clinical and Laboratory Standard Institute (Kalantar et al 2008) using the following antibiotics (Table 1):

Table 1: Antibiotic susceptibility pattern of gram positive bacterial Isolates

Antibiotics used	Antimicrobial Sensitivity Pattern (%) (Mean ±SD value)			
	Coagulase negative Staph sp. (n=10)		*Staphylococcus aureus (n=8)*	
	S	R	S	R
Gentamycin	90±1	10±1	88±1	12±0.57
Co-trimoxazole	33±0.26	67±0.58	50±0.47	50±0.26
Linezolid	99.86±0.2	**	99.76±0.32	**
Levofloxacin	99.80±0.26	**	60±0.1	40±0.2
Tetracycline	99.80±0.26	**	85±0.37	15±0.25
Chloramphenicol	86±0.26	14±0.20	88±0.1	12±0.49
Penicillin G	10±0.1	90±0.3	17±0.1	83±0.1
Cefoxitin	99.66±0.49	**	99.66±0.49	**
Ciprofloxacin	89±0.15	11±0.15	71±0.15	29±0.36
Azithromycin	70±0.1	30±0.2	25±0.1	75±0.2
Erythromycin	99.76±0.32	**	50±0.1	50±0.2
Clarithromycin	99.73±0.30	**	63±0.15	17±0.25
Clindamycin	99.80±0.20	**	75±0.32	25±0.25
Oxacillin	99.70±0.36	**	99.86±0.15	**
Minocycline	90±1	10±1	99.73±0.30	**
Vancomycin	33±0.26	67±0.58	86±0.56	14±0.40
Moxifloxacin	99.86±0.2	**	86±0.30	14±0.61
P- Value	0.000	0.000	0.000	0.001

***: No resistance; S: Sensitive; R: Resistance*

Imipenem, Meropenam, Levofloxacin, Amikacin, Piperacillin/ Tazobactam, Ampicillin / Sulbaclam, Co- Trimaxazole, Cefepime, Amoxyclav, Cefotaxime, Ceftriaxone, Cefuroxime, Tobramycin, Gentamycin, Ampicillin, Cefazollin,. Chloramphenicol, Ceftazidime, Tetracycline, Aztreonam, Pencillin G, Amikacin Linezolid, Moxifloxacin, Cefoxitin, Azithromycin, Erythromycin, Clarithromycin, Clindamycin, Co-Trimaxazole, Oxacillin: Minocycline, and Ciprofloxacin. Statistical

analysis was performed using SPSS (Version 22) by *p* value.

RESULTS:

A total of 295 bacteremia suspected patient's blood culture samples were processed regularly from January 1, 2017 to June30, 2017. Of these 188patients, 62% were females and 38% were males. The median age of patients was 30.5 months with an age range 1 year to 60 years.

Of these total, 9.15% patients had blood culture positive for bacteria in which 6 pathogens were isolated. About 268 specimens yielded no microbial growth. Among the 27 positive cultures, 16 were from males and 11 were from females. The frequency of culture positive cases with respect to different age groups and sex are exhibited in Table 2.The highest percentage of patients 195 (66.10%) were lower than twenty years of age group.

Table 2: Frequency of positive culture with respect to age groups

S.No.	Age Groups (Years)	Sex	No of samples (n)	No. of Positive Cultures (%)	Pattern of Positive Cultures(n)	
					Monomicrobial	Polymicrobial
1.	1- 20	M	121	14	14	-
		F	74	7	7	-
2.	21-40	M	26	1	1	-
		F	10	2	2	-
3.	41-60	M	36	1	1	-
		F	28	2	2	-
Mean Age: 30.5		Total: 295		27 (9.15%)	27	0

Results obtained from different blood samples indicated no uniformity in microbes from different samples. The experimental results also portrayed all infections to be due to single pathogen. The most frequently isolated bacterial pathogens in blood samples are enlisted in Table 3 and Figure 1.

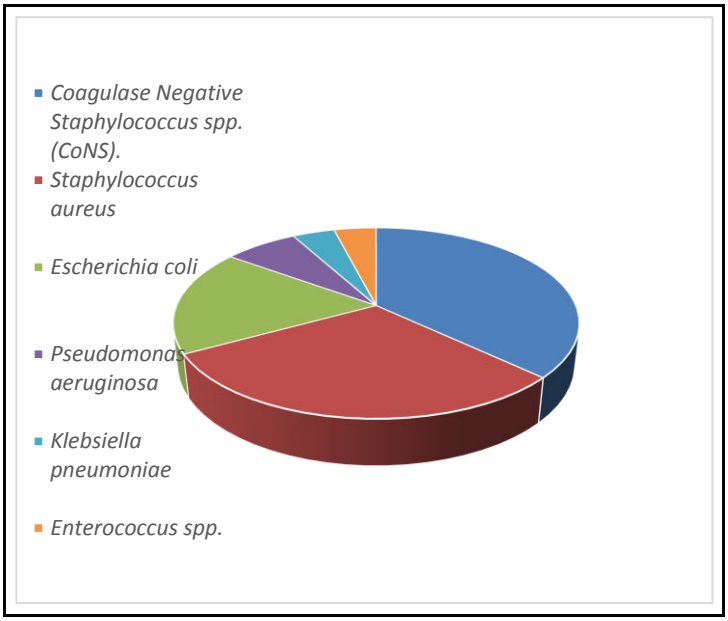

Fig 1. Prevalence of bacterial pathogens in blood samples

Among the 27 isolates 6 genera were identified and the predominant bacteria from the blood culture was Coagulase negative *Staphylococcus* (CoNS) (37%), followed by *Staphylococcus aureus* (30%), *Escherichia coli* (18%), *Pseudomonas aeruginosa* (7%), *Klebsiella pneumoniae* (4%), and *Enterococcus* spp. (4%).Coagulase negative *Staphylococcus*(CoNS) and *Staphylococcus aureus* alone accounted to 67%. The Gram positive and Gram negative bacteria constituted 67% and 33% respectively (Table 3). The predominant pathogens noted were CoNS (64%) followed by *S.aureus* (36%) in the age group between 1 and 20 (Table 4)

Table 3. Prevalence of bacterial pathogens in blood samples

S. No.	Organisms Isolated	Total Pathogens Isolated (%)
1.	**Coagulase negative *Staphylococcus* spp.(CNS).**	**37**
2.	***Staphylococcus aureus***	**30**
3.	***Escherichia coli***	**18**
4.	***Pseudomonas aeruginosa***	**7**
5.	***Klebsiella pneumoniae***	**4**
6.	***Enterococcus* spp.**	**4**

Table 4. Frequencies of bacterial pathogens isolated from blood cultures by age groups

S.No.	Age Group	Pathogens Isolated n (%)					
		Gram Positive		Gram Negative			
		CoNS	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*Enterococcus* spp.
1	1- 20	64	36	57	29	0	14
2	21 - 40	33	67	0	0	0	0
3	41- 60	0	100	50	0	50	0

Antibiotic Susceptibility Patterns

In vitro antibiotic susceptibility of the bacterial isolates (Table 5) exhibited resistance for gram positive bacteria from 10% to 90%. The isolates CoNS were resistant to antibiotics such as Penicillin G (90%), Co-trimoxazole and Vancomycinn (67.23%), Azithromycin (30%), Chloramphenicol (14%), Ciprofloxacin (11%), Gentamycin (10%) and Minocycline (10%). The second predominant pathogen *S. aureus* also showed resistance to Penicillin G (83%), Azithromycin (75%), Co-trimoxazole and Erythromycin (50%), Levofloxacin (40%), Ciprofloxacin (29%), Clindamycin (25%), Clarithromycin (17%), Tetracycline (15%), Vancomycin and Moxifloxacin (14%), Chloramphenicol (12%) and Gentamycin (12%) each.

All gram-positive isolates CoNS were sensitive to Linezolid (99.86%), Moxifloxacin (99.86%), Levofloxacin (99.80%), Tetracyclin (99.80%, Clindamycin (99.80%) Erythromycin (99.76%), Clarithromycin (99.73%), Oxacillin (99.70%), Cefoxidin (99.66%), Cefoxitin (99.66%), Gentamycin and Minocycline (90%), Ciprofloxacin (89.03%), Chloramphenicol (86.10%), Azithromycin (70%), Co-trimoxazole (33.20%) and Penicilin G (10%) whereas the gram positive isolates *S. aureus* were sensitive to Oxacillin (99.86%), Linezolid (99.76%), Minocycline (99.73%), Cefoxitin (99.66%), Gentamycin and Chloramphenicol (88%) Vancomycine (86.16%) Moxifloxacin (86.06%), Tetracycline (85.16%), Clindamycin (75.16%), Ciprofloxacin (71.03%), Clarithromycin (63.03%), Levofloxacin (60%), Erythromycin (50 %), Azithromycin (25%) and Penicillin G (17%). Comparatively, high resistance was observed by the gram positive isolates to Penicillin G (90.90%). Linezolid was an effective antibiotic for gram positive bacteria next to Oxacillinin (Table 5).

The results of susceptibility testing of Gram-negative isolates from blood cultures are summarized in Table 6. Antimicrobial resistance levels for the Gram-negative organisms that cause BSI ranged from 20.30% to 99.86%. *E. coli* was resistant to, Ciprofloxacin (99.7%),

Table 5: Antibiotic susceptibility pattern of gram negative bacterial isolates

Antibiotics used	Antimicrobial Sensitivity Pattern (%) (Mean ±SD value)							
	E. coli (n=5)		*P. aeruginosa* (n=2)		*K. pneumoniae* (n=1)		*Enterococcus* sp.(n=1)	
	S	R	S	R	S	R	S	R
Gentamycin	99±1	**	50±1	50±1	99±1	**	**	100±1
Tobramycin	99.98±0.02	**	50±0.36	50±0.58	99.70±0.36	**	*	*
Cefazolin	39.98±0.20	60.02±0.23	*	*	**	99.66±0.49	*	*
Ampicillin	40±0.26	60±0.25	*	*	**	99.70±0.43	**	99.73±0.37
Meropenem	99.69±0.51	**	99.73±0.30	**	99.73±0.25	**	*	*
Amikacin	99.70±0.43	**	50±0.56	50±1	99.79±0.33	**	*	*
Imipenem	99.95±0.05	**	99.50±0.45	**	99.56±0.51	**	*	*
Ampicillin + Sulbactam	60±0.49	40±0.41	*	*	99.83±0.20	**	*	*
Co-trimoxazole	99.90±0.1	**	*	*	99.73±0.30	**	*	*
Cefuroxime	33±0.1	67±0.26	*	*	**	99.86±0.15	*	*
Piperacillin +Tazobactam	99±1	**	99.86±0.15	**	99.66±0.35	**	*	*
Linezolid	*	*	*	*	**	**	99.80±0.2	**
Amoxycillin + Clavulanic acid	49.96±0.15	50.04±0.2	*	*	**	99.83±0.20	*	*
Ceftriaxone	50±0.1	50±0.3	*	*	**	99.86±0.15	*	*
Levofloxacin	80±0.25	20±0.15	99.6±0.45	**	99.83±0.20	**	*	*
Tetracycline	67±0.15	33±0.15	*	*	99.66±0.35	**	**	99.60±0.36
Chloramphenicol	99.86±0.15	**	*	*	99.83±0.20	**	*	*
Cefepime	67±0.15	33±0.10	50±0.47	50±0.15	**	99.76±0.32	*	*
Cefotaxime	67±0.15	33±0.15	*	*	**	99.80±0.26	*	*
Ceftazidime	60.04±0.75	39.96±0.15	99.76±0.32	**	99.7±0.36	**	*	*
Aztreonam	60±0.1	40±0.2	50.04±0.15	49.96±0.15	**	99.66±0.49	*	*
Penicillin G	*	*	*	*	*	*	**	99.66±0.49
Cefoxitin	99.76±0.25	**	*	*	*	*	*	*
Ciprofloxacin	**	99.7±0.36	*	*	*	*	*	*
P- Value	0.000	0.000	0.001	0.002	0.000	0.003	0.328	0.043

Cefuroxime (67.10%), Ampicillin (60.03%), Cefazolin (59.96%), Amoxicillin + Clavulanic acid (50%) and Ceftriaxone (50%), Ampicillin + Sulbaclam (40.13%) Ceftazidime (39.96%), Aztreonam (40%), Cefepime (33.08),

Tetracycline (33.03%), Cefotaxime (33.03%) and Levofloxacin (20.03%) respectively. *P.aeruginosa* was resistant to Gentamycin (50%), Tobramycin (50.23%), Amikacin (50%), and Cefepime (50.33%) and Aztreonam (49.96%).

Resistant for *K.pneumoniae* was observed against to Cefuroxime, Ceftriaxone, Amoxicillin + Clavulanicacid, Cefotaxime, Cefepime, Ampicillin, Cefazolin and Aztreonam (100%), and for *Enterococcus* spp., it was against Gentamycin, Ampicillin, Tetracycline and Penicillin G (100%). Overall, the study portrayed that Levofloxacin, Tetracycline, Linezolid, and Cefoxitin was fairly effective against both Gram positive and Gram negative isolates.

DISCUSSION:

Bloodstream isolates are the best organisms for the study of antimicrobial susceptibility among human bacterial pathogens as suggested by world surveillance reports (Kalantar*et al*., 2008). With this background, a survey was attempted to monitor the frequency of isolation and evaluate their resistance to antimicrobial agent's patients with bacteremia from Kanyakumari District of Tamilnadu, India since the data on BSI are scant. The study reflected a blood culture positivity rate of 10.6% which was low as compared to those as reported by Vijaya Devi *et al* (2016) with a culture positive rate of 12.4%. The positive cultures reported in the current study focuses only monomicrobial growth (Table 2). The polymicrobial growth isolation rate was 0%.Generallypolymicrobial isolation rate varies between 1 to 15 percent. The polymicrobial growth could have its genesis from contamination or a severe infection with bad prognosis (Chaudhry *et al*., 2000)

Absence of gram-positive rods like *Diphtheroid* spp., *Bacillus* spp. and other gram-positive rods in the present study vividly indicate that the microbiological tests were performed 100% clinical features as these organisms are considered to be strong contaminants arising from to skin contamination at the time of collection or due to contaminated containments (Prakash *et al*., 2011). Currently, 67% of infections were caused by gram-positive and 33% by gram-negative bacteria. Similar reports have been made by Gray (2004) from India. The predominance of Coagulase-negative *Staphylococci* followed by *Staphylococcus aureus* from blood stream infections of suspected patients corroborate with the works of Mucheye Gizachew *et al*. (2013).These organisms are well-known for the cause of community-acquired infections, but there is increasing interest in its role in the epidemiology of hospital-acquired infection too (Opota *et al*., 2015).

The data ascertained from surveillance efforts fortify as an essential component to the design of empirical approaches for the therapy of serious infections and also to redefining appropriate control measures for antimicrobial-resistant pathogens (Michael, 1998; Sader, 2001).Presently, CoNS, *S. aureus*, *E.coli*, *P. aeruginosa*, *K. pneumoniae*, and *Enterococcus* spp. were identified as common bacterial pathogens causing BSI which corroborate with the cases of bacteremia from different countries with varied pathogens on the basis of their proportion and predominance (Reynolds *et al*., 2004; Prakash *et al*., 2011). Several investigations have also documented an increasing frequency of infections due to

coagulase-negative *Staphylococci (*Michael *et al*., 1998; Prakash *et al*., 2011; Lincoln *et al*., 2012).

The resistance of coagulase-negative *Staphylococci* and *S. aureus* to commonly used antibiotics such as Penicillin and Vancomycin was high, but was low towards Chloramphenicol. Favorably, these two gram positive organisms were highly sensitive to most of the antibiotics tested except Penicillin G, Vancomycin and Co-trimoxazole. Thus, efforts should therefore be concentrated on training staff on collecting blood from patients using aseptic precautions.

CONCLUSION:

Currently BSI's are associated with high mortality and increased health care costs. Thus, the present study provides a profile of bacterial pathogens and its antibiotic susceptibility from BSI. This data may assist the technician in the selection of empirical antimicrobial treatment of BSI cases. However, the results of this study cannot be generalized for assisting an empirical therapy of BSI. Extensive studies in patients from multiple medical centers are required to throw light on the epidemiology of infectious diseases, and understand their resistance patterns.

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Acknowledgements

The author would like to thank Mrs. S. Lalitha Srinivasan M.Sc., M.Phil. (Microbiologist), Mrs. P. Jagatha M.Sc., M.Phil. (Quality Manager), Mrs.R.Vigila Christy, M.Sc., M.Phil., (Microbiologist), Prof. Dr. C. Ratnam Nadar, Ph.D., Prof. Dr. S. Ramesh, Ph.D., Mrs. A. Padma, B.Sc. MLT (Lab Technician) and other technicians of our institution for their help in the manuscript preparation, data collection and sampling

CONFLICT OF INTEREST: Authors declared no conflict of interest