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A Study on Community Acquired Bloodstream Infections and Molecular Characterization of Resistant Pathogens in a Tertiary Care Hospital

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A B S T R A C T

Community-acquired bloodstream infections are the infections detected within 48 hours of hospitalization, showing positive blood culture and develop spontaneously without an association with any prior medical interventions. Aims of the study are to identify the clinical profile of patients, detect the pathogens causing community-acquired bloodstream infections (CA-BSI) and their antimicrobial susceptibility pattern and to perform the molecular characterization of resistant pathogens. Under strict aseptic precautions, blood samples were collected and processed as per standard protocol and isolates identified. Their antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method under CLSI guidelines. Vancomycin sensitivity tested using Vancomycin Screen agar and confirmed by E-strip test. Resistant strains were characterized by PCR. Blood culture in 150 patients, detected 12 patients (8%) with CA-BSI. Gram-positive organisms 58% (MSSA 85.7% and 14.3% MRSA) isolated, were highly sensitive to Erythromycin, Vancomycin, Linezolid and 42% Gram-negative organisms (Escherichia coli 60% which were ESBL producers, 20% *Acinetobacter baumannii* and 20% Pseudomonas aeruginosa) isolated, were highly sensitive to Amikacin, Tetracycline each 100% respectively. *bla TEM* and *bla CTX-M* genes among ESBL producers and *mecA* gene in MRSA isolate were positive by PCR. CA-BSI are rising as a major health problem in the upcoming years due to the emergence of antimicrobial resistant strains in the community as well, like ESBL producers, MRSA, etc. Hence, proper surveillance, the framing of appropriate antibiotic policy and preventive strategies curtails the spread of these resistant strains in the community.

Introduction

Bloodstream infections are one of the serious and life-threatening clinical conditions leading to deleterious consequences with a mortality rate ranging from 20-40 %.[1, 2] Hence, needs immediate attention and treatment. Advances in blood culture techniques have resulted in efficient and reliable methodologies for the detection of causative pathogens. Bloodstream infections are classified traditionally as nosocomial and community-acquired bloodstream infections.[3,4] Community-acquired bloodstream infections refers to the infections detected within 48 hours of hospitalization, showing positive blood culture and develops spontaneously without an association with any prior medical

interventions.[5] Community-acquired bloodstream infections are becoming a major health problem in the upcoming years due to the emergence of antimicrobial resistant organisms in community settings as causative agents like, ESBL producing Enterobacteriaceae, Methicillin-resistant *Staphylococcus aureus*.[6] Antimicrobial resistant strains once confined to hospital settings are now a potential threat in the community too. Rapid detection of antimicrobial-resistant strain is highly essential, as they are associated with increased mortality and morbidity and due to their high propensity to spread and able to cause a serious threat to public health concern. Phenotypic characterization of microorganisms helps in identification of causative agents of infectious diseases. Molecular characterization of resistant pathogens aids in tracking the spread of antimicrobial resistance in community and hospital settings.

To identify the clinical profile of patients, detect the pathogens causing community-acquired bloodstream infections and their antimicrobial susceptibility pattern and to perform the molecular characterization of resistant pathogens.

Materials and Methods

Ethical clearance was obtained from the Institutional Ethics Committee before starting the study. This is a cross-sectional study done for a period of 1 year (from March 2017-February 2018) at tertiary care centre, chennai, where blood samples from 150 febrile adult patients with suspected sepsis admitted within 48hrs in Medicine wards, Intensive Care Unit and Surgical wards were collected under strict aseptic precautions and were processed as per standard protocol. The isolates were identified based on Gram stain, colony morphology, and various biochemical reactions. Antimicrobial susceptibility testing for isolated organisms done on Mueller Hinton agar plate by Kirby- Bauer disk diffusion method under CLSI guidelines. Using the differential disk, Cefoxitin (30µg), *Staphylococcus aureus* isolates were categorized into methicillin sensitive and methicillin-resistant strains.[7]

Cefoxitin(30µg)	Susceptible	Intermediate	Resistant
Zone size	>=22mm	-	< or =21mm

Vancomycin sensitivity was tested using Vancomycin screen agar (BHI agar with 6µg/ml of Vancomycin), where 10µl of bacterial suspension was spot inoculated onto this media and incubated overnight at 37℃ along with appropriate controls.[7] After 24hrs of incubation, the sensitivity pattern was interpreted as follows-

-If no visible growth at spot inoculated site-reported as sensitive to Vancomycin.
-If visible growth (> 1 colony) at spot inoculated site was present –reported as resistant to Vancomycin.

E-test procedure

Using an inoculating loop, 4-5 isolated colonies of Staphylococcus were transferred to a test tube containing peptone water and emulsified. Incubated it for 2-4hrs until the growth equal to a 0.5 McFarland turbidity standard was reached. A sterile cotton swab was dipped into this inoculum suspension and pressed against the inside wall of the tube to remove excess fluid and then streaked over the entire surface of Mueller Hinton agar plate evenly in three directions. The surface of agar was allowed to dry completely, and then an E-strip was applied to the agar surface with the MIC scale facing upwards. The plate was then incubated at 37℃ for overnight incubation. After 24 hrs of incubation, the MIC value was read at a point where the edge of inhibition ellipse intersects the strip.

Vancomycin	Susceptible	Intermediate	Resistant
MIC(µg/ml)	< or =2	4-8	>=16

Among the Gram-negative organisms identified, ESBL producers detected as follows-

An initial screening test is done by disk diffusion method under CLSI guidelines using Cefotaxime (30µg) disk and Ceftazidime (30µg) disk which was applied on to Mueller Hinton agar plate inoculated with the test organism and incubated at 37℃ for 24hrs. Screening test denoted ESBL production if zone size was as follows-

Cefotaxime(30µg)	< or =27mm
Ceftazidime(30µg)	< or =22mm

The phenotypic confirmatory test is done by disk diffusion method under CLSI guidelines by the combination disk test method using cefotaxime (30µg) disk and cefotaxime-clavulanic acid (30µg/10µg).

Combination disk test

Disks containing cephalosporin alone and in combination with clavulanic acid were applied onto Mueller Hinton agar plate inoculated with test organism and incubated at 37℃ for 24hrs.

Molecular methods

Characterization of resistant bacterial isolates

The polymerase chain reaction was performed to detect the resistant genes. It included the following steps –

1. Extraction of DNA from all resistant isolates done using *PureFast® Bacterial DNA minispin purification kit*
2. PCR amplification of DNA -using following components Master mix(2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2, 1µl of 10mM dNTPs mix and Red Dye PCR additives)-10µl, primer mix (blaTEM gene Primer mix-260bp, blaCTX-M gene Primer mix-295bp, mecA gene Primer mix-220bp)-5µl and extracted purified DNA-5µl

The PCR products were analyzed using agarose gel electrophoresis, and the sizes of the PCR products were determined by comparing with the DNA ladder ranging from 100bp lower range till 1500bp higher range.

Results and Discussion

The study group included 150 patients in the age group > 18yrs with clinical suspicion of sepsis admitted within 48hrs in Medical, Surgical wards and Intensive Care Units. Blood culture performed in 150 patients, detected 12 patients (8%) with community-acquired bloodstream infection. The majority (n-150) presented with fever predominantly followed by next common presentations were cough/dyspnoea, abdominal pain/ vomiting, dysuria, bleeding disorders/Malena (Fig. 1).

Both Gram-positive and Gram-negative organisms were isolated. 58% of Gram-positive organisms were isolated which included [Methicillin sensitive *Staphylococcus aureus* (MSSA) 85.7% and 14.3% Methicillin-resistant *Staphylococcus aureus* (MRSA)]. 42% of Gram-negative organisms were isolated which included [*Escherichia coli* 60% which were ESBL producers, 20% *Acinetobacter baumannii* and 20% *Pseudomonas aeruginosa*] (Tables 1 and 2). Gram-positive organisms were found highly sensitive to Erythromycin, Vancomycin and Linezolid each 100%

respectively (Table 3). MSSA was found to be highly resistant to Cotrimoxazole (66.7%) followed by Ciprofloxacin (50%). MRSA was found to be highly resistant to Cotrimoxazole, Tetracycline, and Penicillin each 100% respectively. Gram-negative organisms were found highly sensitive to Amikacin, Tetracycline and Imipenem each 100% respectively (Table 4). *Escherichia coli* showed a high level of resistance to Ceftazidime, Cotrimoxazole, Cefotaxime, Ampicillin, and Ciprofloxacin each 100% respectively. *Pseudomonas aeruginosa* exhibited a high level of resistance to Ceftazidime (100%).

Acinetobacter baumannii was also found resistant to Ceftazidime, Ciprofloxacin, Cotrimoxazole, Gentamicin each 100% respectively. Percentage of resistant strains among Gram-positive organisms constituted about 14.3%, and among Gram-negative organisms, the percentage of resistant strains identified was about 60%. *bla TEM* and *bla CTX-M* genes were positive among ESBL (Extended Spectrum Beta-lactamase) producing *E. coli* isolates and *mecA* gene positive in MRSA (Methicillin-Resistant *Staphylococcus aureus*) isolate, by PCR (polymerase chain reaction) (Table 5 and Fig. 2).

Table.1 Gram-positive organisms

Organisms	No. Isolated	Percent
***Staphylococcus aureus* (MSSA)**	6	85.7
***Staphylococcus aureus* (MRSA)**	1	14.3
Total	7	100

Table.2 Gram negative organisms

Organisms	No. Isolated	Percent
E. coli	3	60
Pseudomonas aeruginosa	1	20
Acinetobacter baumannii	1	20
Total	5	100

Table.3 Antimicrobial susceptibility pattern among Gram positive organisms

Organism	**Methicillin Sensitive *Staphylococcus aureus* (MSSA)**		**Methicillin Resistant *Staphylococcus aureus* (MRSA)**	
Number Isolated	6		1	
Drugs	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Ciprofloxacin	50	50	100	0
Penicillin	100	0	0	100
Cotrimoxazole	33.3	66.7	0	100
Erythromycin	100	0	100	0
Linezolid	100	0	100	0
Tetracycline	100	0	0	100
Vancomycin	100	0	100	0

Table.4 Antimicrobial susceptibility pattern of Gram negative organisms

Organism	***Escherichia coli***		***Pseudomonas aeruginosa***		***Acinetobacter baumannii***	
No. Isolated	3		1		1	
Drugs	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Amikacin	100	0	100	0	0	100
Gentamicin	33.3	66.7	100	0	0	100
Ciprofloxacin	0	100	100	0	0	100
Cotrimoxazole	0	100	--------		0	100
Ampicillin	0	100	-------		-------	
Cefotaxime	0	100	-------		-------	
Cefotaxime-clavulanic acid	100	0	-------		-------	
Ceftazidime	0	100	0	100	0	100
Tetracycline	100	0	-------		100	0
Piperacillin-Tazobactam	----------		100	0	100	0
Imipenem	----------		100	0	100	0

Table.5 Molecular identification of antimicrobial resistant genes by PCR

Resistant strains	**Primers**	**Result**
ESBL Producers(3)	blaTEM	POSITIVE
ESBL Producers(3)	blaCTX-M	POSITIVE
MRSA Strain(1)	mecA	POSITIVE

Fig.1 Clinical profile of patients with suspected sepsis (N=150)

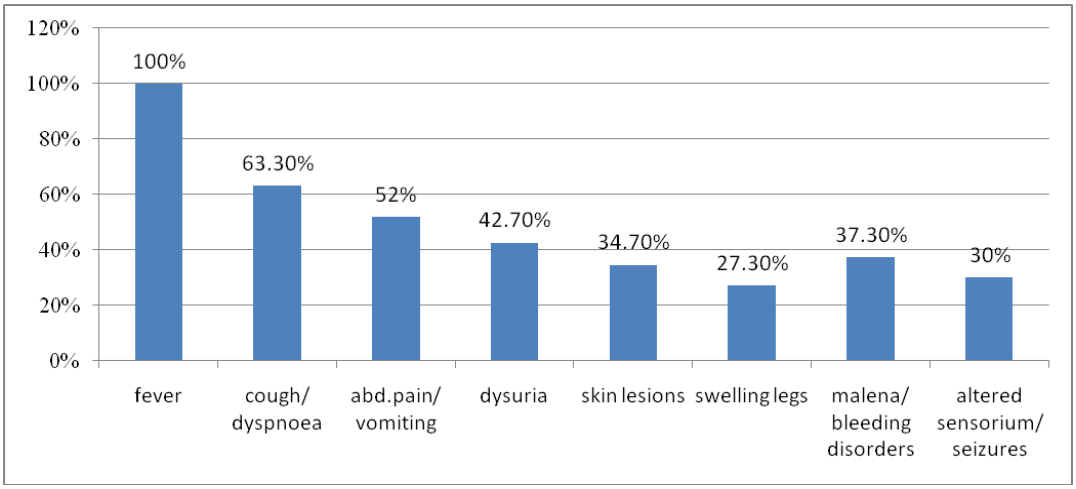
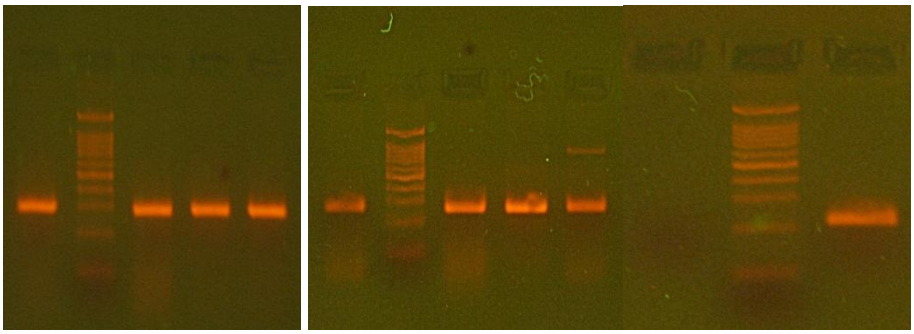


Fig.2 1. bla CTX-M-ladder 2. bla TEM-LADDER 3. NTC-Ladder-mecA



Bloodstream infections are an important cause of mortality and also morbidity related to sepsis. This study was focussed on knowing the burden of community-acquired bloodstream infections in our settings and the pathogens responsible for it.

During the study period of 1 year from March 2017- February 2018, blood culture was done in 150 patients with clinical suspicion of sepsis within 48hrs of hospital admission. Out of which, community-acquired bloodstream infection was detected in 12 patients (8%), in this study. Tufail Soomro *et al.,* [8] (2016) concluded in their study that the frequency and incidence of community-acquired bloodstream infection was 7.6%. Sigauque *et al.,* [9] in their study had identified community-acquired bloodstream infection in 8% of patients on hospital admission correlating well with our study. In a cohort study of 3901 patients with community-acquired sepsis conducted by Nathan I. Shapiro *et al.,* [10] the incidence of bloodstream infection at hospital admission was 8.2%.

In the present study, out of 12 patients with community-acquired bloodstream infection, the frequency and distribution of pathogens were 58% Gram-positive organisms and 42% Gram-negative organisms.

Among the Gram-positive organisms, 85.7% were methicillin-sensitive *Staphylococcus aureus* (MSSA), and 14.3% were methicillin-resistant *Staphylococcus aureus* (MRSA).

Hence, among the Gram-positive organisms, 14.3% were found to be resistant pathogens. In the study conducted by Goncalves- Pereira *et al.,* [11] also the predominant Gram-positive organism isolated were methicillin-sensitive *Staphylococcus aureus* and the predominant Gram-negative organisms identified were *Escherichia coli*. In a study done by Klevens *et al.,* [12] incidences of community-associated methicillin resistant *Staphylococcus aureus* infection was found to be 14%.

In this study, among the Gram-negative organisms, *Escherichia coli* contributed 60%, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* each contributed 20% respectively. The study by Parkins MD *et al.,* [13] also showed that the incidence of community-acquired bloodstream infection cases caused by *Pseudomonas aeruginosa* were 21%, well correlates with our study. Also, in a study of Chung-Ting Chen *et al.,*[14] (2017), *Acinetobacter baumannii* isolates were identified as the causatives of community-acquired bloodstream infections and for these isolates, respiratory tract was the primary source involved which matches with the present study where the *Acinetobacter baumannii* isolate identified was acquired from respiratory tract as primary source of infection. Among the Gram-negative organisms isolated, 60% were found to be resistant pathogens especially, extended-spectrum beta-lactamase (ESBL) producers among *Escherichia coli* organisms. Quan *et al.,* [15] (2017) study revealed 56% of ESBL producing *E. coli* isolates were identified in community-acquired bloodstream infections.

In the present study, among Gram-positive organisms isolated, methicillin-sensitive *Staphylococcus aureus* were highly sensitive to Penicillin (100%), Erythromycin (100%), Tetracycline (100%), Linezolid (100%), Vancomycin (100%) and were resistant to Cotrimoxazole (66.7%) and Ciprofloxacin (50%). Methicillin-resistant *Staphylococcus aureus* was highly sensitive to Ciprofloxacin (100%), Erythromycin (100%), Linezolid (100%), Vancomycin (100%) and was highly resistant to Penicillin (100%), Cotrimoxazole (100%) and Tetracycline (100%).

Among the Gram-negative organisms isolated, *Escherichia coli* isolates were highly sensitive to Amikacin (100%), Tetracycline (100%) and were found highly resistant to Ciprofloxacin, Cotrimoxazole, Ampicillin, Cefotaxime each 100% respectively and Gentamicin (66.7%). *Pseudomonas aeruginosa* was highly sensitive to Amikacin (100%), Gentamicin (100%), Ciprofloxacin (100%), Piperacillin-Tazobactam (100%), Imipenem (100%) and were highly resistant to Ceftazidime (100%). *Acinetobacter baumannii* isolate was highly sensitive to Tetracycline (100%), Piperacillin-Tazobactam (100%), Imipenem (100%) and were highly resistant to Amikacin (100%), Gentamicin (100%), Ciprofloxacin (100%), Cotrimoxazole (100%) and Ceftazidime (100%). Molecular characterization of resistant isolates was done using polymerase chain reaction (PCR) which showed the presence of *bla TEM* and *bla CTX-M* genes, that confirmed ESBL producers among the *Escherichia coli* isolates and similarly, the presence of mecA gene confirmed methicillin-resistant *Staphylococcus aureus*. Luzzaro *et al.,*[16] in his study found that the most prevalent ESBL producing Gram-negative organism was found to be *Escherichia coli* and TEM- type ESBLs were found to be the most prevalent enzymes (45.4%). According to the study by Rossolini *et al.,*[17] the CTX-M-type ESBLs had undergone a rapid and global spread in Enterobacteriaceae recently. In Mario Tumbarello *et al.,* [18] study, the predominantly isolated ESBL genes were bla CTX-M (36.5%) followed by bla TEM gene (28.7%). Nagat Sobhy *et al.,* [19] study emphasized that

the identification of the *mecA* gene is the most reliable method for detecting the MRSA isolate.

In conclusion, community-acquired bloodstream infections are rising as a major health problem in upcoming years due to the emergence of antimicrobial resistant organisms which were once confined to hospital settings are now a potential threat in the community settings as well like ESBL producing Enterobacteriaceae, MRSA, etc. Hence, these antimicrobial resistant strains should be promptly identified through proper surveillance. Molecular characterization of resistant pathogens helps in tracking the spread of antimicrobial resistance in the community. Also, appropriate antibiotic policy and preventive strategies have to be framed to curtail the spread of these antimicrobial resistant strains in the community settings.

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References

1. Pedersen G, Schonheyder H.C., Sorensen H. T. Source of infection and other factors associated with case fatality in community- acquired bacteremia- a Danish population-based cohort study from 1992 to 1997. Clin Microbiol Infect 2003; 9:793-802.
2. Larry G. Reimer, Michael L. Wilson, Melvin P. Weinstein. Update on detection of bacteremia and fungemia. Clinical Microbiology Reviews, 1997, 10:444-465.
3. J. Rodriguez- Bano, M.D. Lopez-Prieto, M.M. Portillo, *et al.*, Epidemiology and clinical features of community- acquired, healthcare-associated and nosocomial bloodstream infections in tertiary-care and community hospitals. Clin Microbiol Infect 2010; 16: 1408-1413.
4. Garner JS, Jarvis WR, Emori TG, *et al.*, CDC definitions for nosocomial infections. Am J Infect Control 1988; 16:128-40.
5. Yardena Siegman – Igra, Boaz Fourer, Ruth Orni- Wasserlauf, *et al.*, Reappraisal of community- acquired bacteremia: A proposal of a New Classification for the spectrum of acquisition of bacteremia. Clinical infectious diseases 2002; 34:1431-9.
6. Johann D.D. Pitout, Patrice Nordmann, Kevin B. Laupland *et al.*, Emergence of Enterobacteriaceae producing extended- spectrum β-lactamases (ESBL) in the community. J antimicro chemo. 2005; 56: 52-59.
7. Clinical and Laboratory Standards Institute (CLSI) M100 27th edition Performance Standards for antimicrobial susceptibility testing.
8. Tufail Soomro, Shiyam Sunder Tikmani, Sumera Aziz Ali. Frequency and Etiology of community- acquired bloodstream infection in hospitalized febrile children. J Med Diagn Meth 2016; 5:217.
9. Sigauque B, Roca A, Mandomandol, *et al.*, Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr Infect Dis J. 2009; 28:108-13.
10. Nathan I. Shapiro, Richard E. Wolfe, Sharon B. Wright, *et al.*, Who needs a blood culture? A prospectively derived and validated prediction rule. J Emerg Med 2008; 35:255-264.
11. J. Goncalves Pereira, P.R. Povoa, C.

Lobo, *et al.,* Bloodstream infections as a marker of community-acquired sepsis severity. Results from the Portuguese Community-acquired sepsis study (SACiUCI study). Clin Microbio Infect 2013; 19:242-248.

12. Klevens RM, Morrison MA, Nadle J, *et al.,* Invasive Methicillin resistant *Staphylococcus aureus* infections in the United States. JAMA 2007; 298:1763-1771.
13. Parkins MD, Gregson DB, Pitout JD, *et al.,* Population-based study of the epidemiology and the risk factors for *Pseudomonas aeruginosa* bloodstream infection. Infection 2010; 38:25-32.
14. Chung-Ting Chen, Yung-Chih Wang, Shu-Chen Kuo, *et al.,* Community-acquired bloodstream infections caused by *Acinetobacter baumannii*; A matched case-control study. j. jmii.2017; 02.004.
15. Quan J, Zhao D, Liu L, *et al.,* High prevalence of ESBL- producing *Escherichia coli* and *Klebsiella pneumoniae* in community-onset bloodstream infections in China. J Antimicrob Chemother 2017; 72:273-280.
16. Luzzaro F, M. Mezzatesta, C. Mugnaioli, *et al.,* Trends in production of extended spectrum beta-lactamases among *Enterobacteria* of medical interest: report of the second Italian nationwide survey. J. Clin. Microbiol. 2006; 44: 1659-1664.
17. Rossolini G.M, M.M.D′ Andrea, C.Mugnaioli. The spread of CTX-M-Type extended spectrum β-lactamases. CLIN. Microbiol. Infect. 2008; 14(suppl.1): S33-S41.
18. Mario Tumbarello, Michela Sali, Enrico Maria Trecarichi, *et al.,* Bloodstream infections caused by Extended- spectrum- β-lactamase-producing *Escherichia coli*: Risk factors for inadequate initial antimicrobial therapy. J Antimicrob Chemother. 2008; 52:3244-3252.
19. Nagat Sobhy, Fatma Aly, Ola Abd El Kader *et al.,* Community-acquired methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections(in a sample of Egyptian population): analysis of mecA gene and Staphylococcal cassette chromosome. Braz J Infect Dis.2012; 16:426-431.

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