Innovations

Bacteriological Analysis with Antimicrobial Sensitivity in Blood Culture – A Roadmap for Antibiotic Stewardship Establishment

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Abstract

Problem: *Blood cultures are an extremely important tool that is employed as one of the gold standard tests in the diagnosis and management of nosocomial bacteremia among hospitalized patients. If the laboratory exercise is done with too much caution, then more negative impacts will be recorded in the process of blood culturing.* ***Methodology:*** *The main objective of this study is to determine the etiological profile and antibiotic sensitivity pattern of blood cultures. An observational study was conducted in a tertiary care teaching hospital in Tiruchirappalli, India, where a total of 359 blood samples were cultured bacteriologically and analyzed. Aerobic culture bottles were inoculated with blood samples and incubated in the BACTEC FX40 system till they flashed a positive signal, or for a maximum of 5 days. Positive signal bottles were processed for etiological identification and antimicrobial sensitivity patterns. The subjected blood samples were blood culture samples from clinically suspected bacteremia patients ranging in age from newborn to 91 years.* ***Findings:*** *In our study, we found a male predominance of 62%. Out of which, the 41–50 age group had the highest positivity rate of 29%. Culture-positive cases were 68, which gives a percentage of 19% positivity. While determining the bacterial etiology, Gram-positive cocci and Gram-negative bacilli showed 24 and 76%, respectively; further analysis reported Staphylococcus aureus and Escherichia coli predominated. The antibiotic sensitivity pattern among Gram-positive strains showed high resistance to Penicillin (50%), Clindamycin (46%), Erythromycin (43%), and Ciprofloxacin (43%), while Gram-negative strains showed 41, 37, and 36% resistance to Amoxyclav, Ceftrioxone, and Ciprofloxacin.* ***Conclusion:*** *This study highlighted the importance of bacterial culture auditing, starting from the method of culturing to reporting the etiology and resistance pattern to establish the institutional antibiotic policy and develop stewardship.*

Keywords: *Blood culture, MDR, pattern, Antibiotic stewardship*

Introduction

Bloodstream infections have a wide range of infections, from self-limiting to life-threatening conditions like sepsis, which require fast and aggressive treatment (Timsit et al., 2020). Early detection and identification of microorganisms that are clinically relevant to bloodstream infection is essential. Early detection antimicrobial sensitivity patterns for rapid administration of antimicrobial treatment have shown a reduction in morbidity and mortality in bloodstream infections (Negussie et al., 2015).

Proper knowledge of antimicrobial sensitivity and resistance profiles in the local area and hospital can prevent the irrational use of antibiotics and the spread of antibiotic resistance (Kumar et al., 2013). Blood cultures are one of the important tools that are used for the diagnosis and treatment management of the bloodstream infection patient. Blood cultures are done on clinically suspected patients to find out if the causative organism is bacterial or fungal (Chela et al., 2019). The epidemiological and antimicrobial sensitivity patterns of pathogens isolated in a given locality will enhance treatment for the patient (Ahmed et al., 2014). These studies motivated the design of an objective to determine the etiological profile of the blood cultures, subsequent antibiotic sensitivity, and resistant pattern of the blood cultures.

Materials and Methods

This study was designed as retrospective and observational and was conducted in a tertiary care teaching hospital in Tiruchirappalli, India, for a period of nine months (June 2022 to February 2023). A total of 359 clinically suspected bacteriemia patients whose blood culture specimens were included. The blood culture bottles were inoculated at the bedside, phelobotomy, and laboratory.

All the blood samples were collected from inpatients before antibiotic administration. All the inoculated blood culture bottles were incubated at the BACTEC FX40 system till they flashed a positive signal or until 5 days after, which is considered negative. Once there is a positive signal, Gram's stain and other bacterial identification techniques are done.

Bacterial etiologies were identified, and antibiotic susceptibility patterns were performed as per Clinical Standard Institute and Laboratory (CLSI) Guidelines (CLSI, 2023). *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603 were included as quality control strains. The collected data were analyzed using an Excel spreadsheet and expressed as percentages using descriptive statistics.

Results

We analyzed 359 blood samples from clinically suspected bacteremia patients for culturing with the aid of the Bactec Automated Blood Culture System, where culture positivity was observed among 68 samples (18.9%) (Figure 1). The age group of the culture-positive patients ranged from newborn to 91 years. It was recorded that there was a male predominance of 62% and the highest age group between 41 and 50 years, with a rate of 29% (Figure 2).

The culture pattern revealed vast variations between gram-positive and gram-negative bacterial isolates, where the possible number of isolates was 69. Among the Gram-positive group, cocci alone were isolated and identified as *Staphylococcus aureus, Streptococcus* sp., and *Enterococcus* sp., with an isolation rate of 24%. Conversely, *Escherichia coli, Klebsiella pneumoniae*, *Salmonella enterica* Typhi*, Pseudomonas aeruginosa, Acinetobacter baumani*, non-fermenting GNB, and *Citrobacter freundii* were the possible Gram-negative isolates with an isolation rate of 76% (Table 1). Among the isolates, only one sample showed polymicrobial bacteremia with the combination of *Pseudomonas* and *Enterococcus*, whereas others revealed monomicrobial bacteremia.

The antibiotic sensitivity patterns of Gram-positive and Gram-negative bacterial isolates provided clear information about the antibiotic resistance strain sustainability among patients and indicated that it was neither elevated nor reduced. Among Gram-positive isolates, high resistance was observed to Penicillin (50%), Clindamycin (46%), Erythromycin (43%), and Ciprofloxacin (43%) (Table 2), while Gram-negative strains showed 41, 37, and 36% resistance to Amoxyclav, Ceftrioxone, and Ciprofloxacin (Table 3).

Discussion

As bacteremia and septicaemia are the leading causes of mortality, it is essential to perform bacteriological analysis with antibiogram for an effective management (Thakur et al., 2016). In this study 19% positivity observed which is correlated with studies reported in Ghana (13.1%) and North India (16.5%)

(Deku et al., 2019; Gupta and Kashyap, 2016). Comparatively, lower rates also reported in India (Gohel et al., 2014; Khanal et al., 2020).

The prevalence of blood stream infection (BSI) is found to maintain a male predominance (Akoyu et al., 2015; Thomas et al., 2019); however, no statistical significant association observed between gender and BSI; but there are reports revealed the prevalence of BSI is higher among females (Yasihun et al., 2015; Kumalo et al., 2016). In our study, it was recorded that the least number of positivity rates of 10.3% in newborn to 10 years of age group which is comparatively lower than the studies conducted in Nigeria, Ethipopia, Egypt ranged from 19 to 41% (Ogunkunle et al., 2020; Iregbu et al., 2006; Uzodimma et al., 2013; Sorsa et al., 2019; Shehab et al., 2015).

In our study, the blood samples were collected before the commencement of antibiotic treatment. Unfortunately, prior self-medication at home or use of antibiotics at peripheral health facilities before transfer to our centre could not be completely determined from the history of the patients which have also contributed to the low positivity rate. The diversity of bacterial isolates (Table 1) is in concordance with the findings from some results published from USA and Ethiopia (Orsini et al., 2012; Dagneu et al., 2013).

The culture positivity of Gram positive (24%) and Gram negative (76%) organisms was observed in this study and references also revealed the predominace of gram negative isolates (Gupta and Kashyap, 2016, Lee et al., 2007). In contrast, some studies have reported Gram positive predominance from cases of BSI (Deku et al., 2019, Khanal et al., 2020; Rani et al., 2012; Reddy et al., 2010; Mia et al., 2020; Sangita et al., 2019). The Gram positive bacterial pathogens were found to be dominated etiology for bacteremia and sepsis before introduction antibiotics in the 1950s. Later, the trend was changed and Gram-negative bacterial etiology evolved (Oyekale et al., 2022). In healthcare settings, there is a higher chance of hospital acquired Gram-negative bacterial infections which cause BSI due to the instruments and procedures carried out on these patients.

Out of ten isolates of S. aureus, six were Methicillin resistant *Staphylococcus aureus* where 60% of the *S. aureus* were penicillin resistant. The higher percentage of MRSA strains observed in this study might be due to the frequent use of the penicillin and third-generation cephalosporin as part of the emergency empirical therapy. Several studies also indicated that the frequent use of cephalosporin in hospitals across the globe is correlated to the emergence and spread of MRSA (Dancer, 2001).

The predominant isolate among Gram negative group is Escherichia coli where the isolates showed high resistance of 33% among amoxicillin, amoxicillin-clavulanate, ciprofloxacin, co-trimoxazol and cefotaxime (Table 3). The study which defined the *E. coli* as one of the isolates, showed no resistance against first line antibiotics and showed resistance towards imipenem, clarithromycin and cephalosporin (Khan et al., 2021).

To understand the resistant status of Klebsiella penumoniae, the range was documents from 14 to 57% where high resistant observed among amoxicillin and ceftazidime. A study showed high resistance towards various antibiotics including piperacillin-tazobactum (PIT) but in our study no resistance observed against PIT (Khan et al., 2021). The multidrug resistancy was documented predominantly in Acinetobacter sp where third line drugs and the last resort antibiotic colistin also showed resistance with 50%. Another study revealed the multi drug resistance like amoxicillin-clavulanic acid, ceftazidime, ciprofloaxacin and imipenem (Fox et al., 2018), The Pseudomonas and Salmonella species showed least resistance towards the panel of antibiotics used in this study (Okon et al., 2009; Mayr et al., 2014; Ahmad et al., 2019).

Conclusion

In our study, Gram negative bacteria has occupied as major etiological agent and *E. coli* is most prevalent; where *S. aureus* dominated in Gram positive group. Antibiotic resistances to penicillin and cephalosporins showed higher level thereby regular surveillance and audit of sensitivity patterns, and formulation of hospital antibiotic policies is mandate where carbapenems must be the last resort, in antibiotic treatment, for most facilities in the developing countries, and newer antibiotics are not available for the majority, especially for ESBL-producing Gram-negative bacteria. Based on existing data, and compliance with treatment guidelines, this study may help to curtail irrational antibiotic use and reduce emergence of resistance among bacteria.

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Figure 1: Culture positive status (n=358)

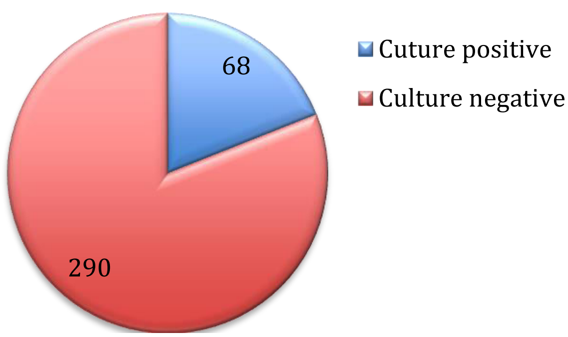

Figure 2: Age and gender wise analysis of culture positive patients

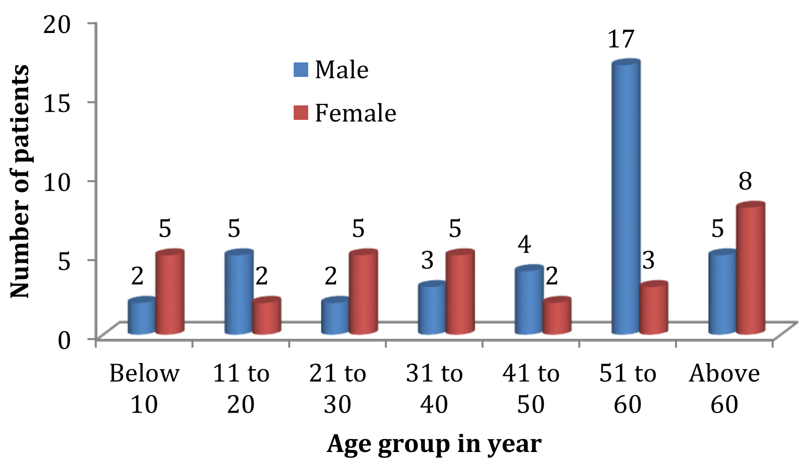

Table 1: Bacteria

l culture pattern

Bacterial isolate	Number of isolates (n=69)	Percentage
Gram Positive		
Staphylococcus aureus	10	14.5
Streptococcus sp	1	1.4
Enterococcus sp	3	4.3
Gram Negative		
Escherichia coli	6	8.7
Klebsiella pneumoniae	14	20.3
Salmonella sp	7	10.2
Citrobacter freundii	1	1.4
Pseudomonas aeruginosa	20	29
Other Non fermenter	3	4.4
Acinetobacter baumani	4	5.8

Table 2: Antibiotic resistance patterns of Gram-positive bacterial isolates

Bacteria isolated	**Percentage of resistance against tested antibiotics**											
	P	**CIP**	**E**	**CD**	**GEN**	**AK**	**DOX**	**LZ**	**RIF**	**COT**	**TEI**	**CX**
Staphylococcus aureus	60	50	20	50	10	10	20	0	10	20	30	60
Enterococcus sp	0	...	...	...	100	...	...	...	...	...	...	0

[P-penicillin, CIP- ciprofloxacin; E-Erythromycin; CD-Clindamycin, GEN - gentamicin; AK-Amikacin,LZ-Linezolid, RIF- Rifampicin, COT - Co-Trimoxazole TEI- Tecolanin

Note: 0 - no resistance; ... shows the antibiotics were not tested against this organism

Table 3: Antibiotic resistance patterns of Gram-negative bacterial isolates

Bacterial isolate	Percentage of resistance against tested antibiotics													
	AMP	**AMC**	**CIP**	**COT**	**GEN**	**AK**	**CAZ**	**CTX**	**CPM**	**PIT**	**IPM**	**AT**	**CX**	**CL**
Escherichia coli	33	33	33	33	0	0	16	33	16	16	16	0	16	0
Klebsiella Pneumoniae	57	35	21	21	14	14	57	35	14	0	14	14	28	0
Salmonella sp	0	0	28	0	…	…	…	0	….	…	0	…	…	…
Pseudomonas aeruginosa	26	26	21	…	…	…	15	…	5	0	0	15	…	0
Acinetobacter sp	25	25	50	50	0	50	50	25	50	25	50	…	…	25

AMP - amoxicillin, AMC - amoxicillin-clavulanate; CIP, ciprofloxacin; COT - co-trimoxazole, GEN, gentamicin; AK-Amikacin, CAZ, ceftazidime; CTX, cefotaxime, CPM, cefepime; PIT, Piperacillin/tazobactam IPM: Imipenem; AT-Aztreonam , CX- Cefoxitin, CL-Colistin
Note: 0 - no resistance; … shows the antibiotics were not tested against this organism