ORIGINAL ARTICLE

Assessment of Bacterial Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates

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ABSTRACT

Objective : To determine the pattern of bacterial isolates in bloodstream infections and their antimicrobial susceptibility in a tertiary care hospital, Lahore.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: The study was carried out at the Pathology department of Combined Military Hospital, Lahore from November 2019 to January 2020.

Materials and Methods: A total of 359 blood culture specimens were collected over a period of three months. Organisms were identified by using API. Antimicrobial susceptibility testing was carried out by Modified Kirby Bauer disk diffusion method on Mueller Hinton agar and interpreted by CLSI guidelines 2019.

Results: Out of 359 bacterial isolates, only 11(3.1%) were Gram-positive cocci, whereas 348 (96.9%) isolates were Gram-negative rods (GNRs). Amongst the GNRs, most commonly isolated organism was *Salmonella typhi* (207; 59.5%) followed by *Salmonella paratyphi* (60; 17.2%). Twenty-seven (7.7%) *Acinetobacter* sp., 20 (5.7%) *E. coli* and 20 (5.7%) *Klebsiella* sp. were isolated. The antimicrobial resistance pattern of *S. typhi* showed 158 (76%) MDR and 106 (51%) XDR isolates.

Conclusion: The emergence of MDR and XDR bacteria especially amongst *Salmonella typhi* is quite daunting. Our study emphasizes the importance of antibacterial susceptibilities surveillance in determining the sensitivity pattern of microorganisms causing Blood stream infections.

Key Words: *Blood Stream Infections, Multidrug Resistance, Blood Cultures.*

Introduction

Infection caused by viable organisms is known as Blood stream infection (BSI).[1] The Clinical scale of BSI ranges from mild bacteremia to severe septic shock.[2] A scientific publication in 2017 reported that sepsis accounted for almost 20% of all global deaths.[3] These infections remain a significant cause of morbidity, mortality, prolonged periods of hospital stay, and higher health care cost worldwide. Increased mortality of BSIs is often attributed to inadequate diagnostic facilities and inappropriate, delayed, or insufficient treatment.[1]

Prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) and methicillin-resistant *Staphylococcus aureus (MRSA)* is on the rise.[4] The occurrence of multidrug-resistant bacteria and the failure to develop new antibiotics has compounded this public health issue.[5]

Despite immense improvements in clinical diagnosis, blood culture remains the gold standard test for BSI detection. The spectrum of microorganisms isolated form hospitals and their antibiotic susceptibilities not only varies according to geography but even within the same hospital setting.[6] This holds true for Pakistan as well, as we have recently seen a shift to a rise in Gram negative bacteria as compared to Gram positive bacteria.

Antimicrobial resistance (AMR) has become a profoundly serious issue in Pakistan, as there is a paucity of a good quality blood stream infection surveillance data that can influence policy change.

The present study was undertaken to determine the pattern of bacterial isolates in blood stream infections and their antimicrobial susceptibility pattern. The aim of the study was to determine the recent trend in antimicrobial susceptibility pattern of microorganisms that cause BSI in our setup and to

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prepare an antibiogram that will aid the clinicians in executing better decisions in treating their patients and can help to improve the antimicrobial stewardship programs in their hospital setting.

Material and Methods

This was a descriptive cross-sectional study done at the Pathology department of CMH, Lahore from November 2019 to January 2020 after getting approval from the Ethical Review Committee of CMH Lahore Medical College (IRB No: 532/ERC/CMH/LMC).

Simple convenient sampling technique was employed.

A total of 359 samples of blood culture from patients with suspected signs of infection that presented to either OPD or indoor facilities of Combined Military Hospital were included in the study. Duplicate samples were excluded. Both adult and pediatric tryptic soya broth blood culture bottles were used to collect blood through aseptic blood collection technique. All the samples were collected before start of any antimicrobial drugs in the hospital. About 5 ml and 1 ml of blood was drawn and then inoculated into the adult and pediatric blood culture bottle.

The blood culture bottles were transported to Microbiology section of Combined Military Hospital Lahore and were placed in an incubator at 35±2 ºC overnight. First subculture from broth bottles was done on Blood agar and MacConkey agar plates. The subculture plates were incubated at 35±2 °C overnight and observed next day for any visible growth. If no growth occurred, then second and third subculture were done at day 4 and 7. The blood culture bottles were incubated for seven days in case of negative subculture.

Preliminary identification was based on Gram staining, catalase test, oxidase test and motility. Catalase positive and oxidase negative rods were identified by analytical profile index (API) 10S (BioMerieux). Oxidase positive rods were identified using API 20NE (BioMerieux).

Gram positive, catalase positive cocci were identified by coagulase and deoxyribonuclease (DNAse) tests. Gram-positive cocci with a negative catalase test were further grouped by Streptococcal grouping latex kit UK. Antimicrobial susceptibility testing was carried out by Modified Kirby Bauer disk diffusion method on Mueller Hinton agar and interpreted by CLSI guidelines 2019. Vancomycin and colistin susceptibility were tested by using E test method and broth microdilution method, respectively as per CLSI guidelines.[7]

Statistical analysis was done by using SPSS 22. Descriptive analysis of sample distribution, age, sex, and antimicrobial data was performed, and results are presented as frequencies and percentages.

Results

The study was conducted over a period of three months and a total of 359 positive blood cultures were collected during this period. Positivity was higher in males (233; 64.9%) as compared to females (126; 35.1%). Majority of the samples with positive culture were isolated from patients visiting the OPD 177 (49%) followed by medical ward 79 (22%), pediatric ward 69 (19%) and 34 (10%) from ICU.

Out of 359 bacterial isolates, only 11(3.1%) were Gram-positive cocci, whereas 348 (96.9%) isolates were Gram-negative rods (GNRs). Amongst the GNRs, most isolated organism was *Salmonella typhi* (207; 59.5%) (Table I).

Table I: Breakup of Gram Positive and Gram-Negative Isolates from Positive Blood Cultures (n=359)

Organism		**Number Isolated**	**Percentage (%)**
Gram Positive Cocci		**11**	**3.1**
	Staph. aureus	04	4/11 = 36.4
	CoNS	05	5/11= 45.4
	E. faecalis	02	2/11= 18.2
Gram Negative Rods		**348**	**96.9**
	Salmonella typhi	207	207/348= 59.5
	Salmonella paratyphi	60	60/348= 17.2
	Acinetobacter sp	27	27/348= 7.7
	Escherichia coli	20	20/348= 5.7
	Klebsiella sp	20	20/348= 5.7
	Pseudomonas sp	05	5/348= 1.4
	Citrobacter	03	3/348= 0.86
	Enterobacter	02	2/348= 5.3
	Serratia	03	3/348= 0.86
	Burkholderia	01	1/348= 0.28

Out of the 11 Gram-positive cocci isolated, 4 were *Staphylococcus aureus* (2 Methicillin sensitive staphylococcus aureus MSSA strains and 2 Methicillin sensitive staphylococcus aureus MRSA strains), 5 were Coagulase negative Staphylococci (CoNS). These were considered as contaminants and not processed further. Two strains of *Enterococcus fecalis* were isolated and were susceptible to vancomycin.

The antimicrobial resistance pattern of *Salmonella typhi* showed that out of the 207 isolates, 158 (76%) were MDR and 106 (51%) were XDR strains. No isolate was resistant to either meropenem or azithromycin (Table II).

Table II: AMR Pattern of *S. Typhi* and *S. Para typhi* Isolates Form Positive Blood Cultures (N=267)

Antibiotics	Salmonella Typhi (N=207)		Salmonella Para Typhi (N=60)	
	Resistance No.	%	Resistance No.	%
Ampicillin	158*	76	14	23
Fluoroquinolones	203†	98	60	100
Ceftriaxone	106**	51	01	2
Meropenem	0	0	0	0
Azithromycin	0	0	Not tested	
Chloramphenicol	163	79	10	17
TMP-SMZ	160	77	14	23

*MDR *S. typhi* isolates (defined as resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole)
**XDR *S. typhi* isolates (defined as resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole, fluoroquinolones and third generation cephalosporins)
† 98% isolates were resistant to fluoroquinolones.

It was observed (Fig.1) that *S. typhi* was more commonly isolated in younger age groups, whereas, *E. coli*, *Acinetobacter* sp. and *Klebsiella* sp. were isolated from older age groups.

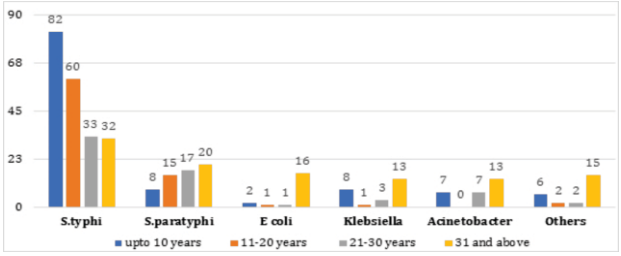

Fig. 1: Distribution of Positive Blood Cultures According to Age Group

Antimicrobial resistance pattern of *E. coli* and *Klebsiella sp*. and *Acinetobacter sp.* is shown in Table III.

Resistance to third generation cephalosporins was 85%, 70% and 52% respectively for *E. coli*, *Klebsiella* and *Acinetobacter sp.* Overall fluoroquinolones were 100% resistant to *E. coli*. Whereas they showed 75% and 52% resistance to *Klebsiella* and *Acinetobacter sp*. respectively.

E. coli, *Klebsiella* and *Acinetobacter sp*. showed no resistance to colistin.

Table III: Antimicrobial Resistance Pattern of E. Coli, Klebsiella Sp. and Acinetobacter Sp. Isolates Form Positive Blood Cultures (n=67)

Antibiotic	Antimicrobial Resistance of Organisms					
	Escherichia coli n=20		Klebsiella n=20		Acinetobacter n=27	
	No	%	No	%	No	%
Amoxicillin-Clavulinic acid	14	70.0	20	100	17	62.9
Third generation Cephalosporins	17	85.0	14	70.0	14	51.8
Imipenem	04	20.0	12	60.0	10	37.0
Meropenem	03	15.0	12	60.0	10	37.0
Fluoroquinolones	20	100	15	75.0	14	51.8
Gentamicin	08	40.0	13	65.0	18	66.6
Amikacin	02	10.0	12	60.0	14	51.8
TMP-SMZ	16	80.0	13	65.0	10	37.0
Doxycycline	19	95.0	14	70.0	15	55.5
Colistin	0	0	0	0	0	0
Tigecycline	11	55.0	14	70.0	13	48.1
Piperacillin-Tazobactam	05	25.0	13	65.0	09	33.3
Cefaperazone-sulbactam	04	20.0	13	65.0	08	29.6

Discussion

The appropriate use of antibiotics by clinicians is paramount in preventing antimicrobial resistance. The challenges faced by the developing world in monitoring antimicrobial resistance are lack of surveillance systems, inadequate means, and indigent compliance to prevention of infection and injudicious prescription as well as use of antibiotics.[8]

In the current study, 96.9% isolates were Gram-negative rods (GNRs) while only 3.1% were Gram-

positive cocci. In another study conducted in Lahore, out of a total of 267 positive blood cultures, 112 (41.9%) cases were of Gram-positive cocci followed by 102 (38.2%) isolates of non-fermenters and 52 (19.47%) isolates were of Enterobacteriaceae.[9] In a study conducted by Kulkarni [10], a total of 720 samples showed growth on culture. 60.67% of the bacterial isolated were Gram-positive whereas 39.33% were Gram-negative bacteria.

The most isolated organism isolated among the GNRs in the present study was *Salmonella typhi* (59.5%) followed by *Salmonella paratyphi* (17.2%). *Acinetobacter* sp.*, E. coli and Klebsiella* sp. were isolated in the frequency of 7.7%, 5.7% and 5.7 % respectively.

A study conducted by Imran et al[9] showed *Staphylococcus aureus* and coagulase negative *Staphylococcus* sp. isolation as 56.25 % and 41.96 % respectively. Whereas, amongst the Enterobacteriaceae, 55.76 % were *E. coli* and *Klebsiella* species were 34.6 %. Among 102 non-fermenters, 68.6 % were *Acinetobacter* sp. and 31.37 % were *Pseudomonas* sp.

The antimicrobial resistance pattern of *S. typhi* showed 158 (76%) MDR and 106 (51%) XDR isolates. No isolate was resistant to either meropenem or azithromycin. This is comparable to a study conducted by Hussain et al [11] in which isolation of multidrug-resistant (MDR) isolates was 76% in *Salmonella typhi* and 34% in *Salmonella paratyphi*. One hundred and fifteen (48%) isolates of *Salmonella typhi* were Extensively drug resistant.

Another study conducted in Rawalpindi [12] showed isolation of MDR isolates of *S. typhi* to be 57% whereas in case of *S. paratyphi* A, it was 42%. Ninety-eight percent strains of *S. typhi* were resistant to fluoroquinolones, a finding supported by regional studies as well as in India and Bangladesh. A study conducted by Shrestha[13] in Nepal also showed 94.6% resistance to fluoroquinolones among Salmonella species. The increase of MDR and XDR isolates of *S. typhi* has become one of the serious issues as the clinicians are left with few choices resulting in increased cost of treatment.

The resistance pattern of *Escherichia coli* in the current study was relatively greater in comparison to other studies performed in the region. A study in Port Blair, India[14] showed *E. coli* sensitive to fluoroquinolones in 55.5% isolates, 50% sensitive to ceftriaxone, 90% sensitive to imipenem and 83% to meropenem, 75% sensitive to gentamicin and 90% to amikacin, respectively.

In the current study, the resistant pattern of *Klebsiella* species to third generation cephalosporins, fluoroquinolones, Imipenem and meropenem was comparable to a study in Nepal which showed *Klebsiella* to be highly resistant to third generation cephalosporins, fluoroquinolones and aminoglycosides but showed better susceptibility to Colistin, Carbapenems, and Tigecycline.[15]

Prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) is rising. The present study showed 20% resistance to imipenem, 15% resistance to meropenem in strains of *E. coli* while resistance to imipenem and meropenem were seen in 60% of the isolates of *Klebsiella*. The isolation of carbapenem-resistant *Klebsiella pneumoniae* rose from <0.1% in 2002 to 4.5% in 2010 in the United States.[16]

Acinetobacter species are often multidrug resistant and associated with life threatening infections.[17] *Acinetobacter sp.* isolated in the current study showed significant resistance to third generation cephalosporins, fluoroquinolones, gentamicin, and amikacin. No isolate was resistant to colistin. In a study conducted in Delhi, 80.3% of the isolates of *Acinetobacter* sp. revealed resistance to at least three or more classes of antibiotics.[18]

The present study endorses the importance of antimicrobial surveillance as a valuable means in evaluating the load of AMR. Surveillances on a national scale are essential for providing decision makers with the information they need to develop appropriate action plans. Antibiograms are more helpful for clinicians in making up to date decisions about optimum empirical therapy.

This study emphasizes the need to motivate clinician to request antimicrobial sensitivity testing more frequently for better treatment outcome.

The limitation of our study was that it was a single center study; hence more studies involving multiple hospitals should be carried out so that the results can be more reflective of the AMR issue in our region.

Conclusion

Emergence of MDR and XDR Salmonella along with CRE is quite alarming. Unfortunately, indiscriminate use, easy availability and over the counter use of

antibiotics has compounded the issue of AMR. The present study focuses on the significance of antimicrobial susceptibilities surveillance in determining sensitivity pattern of microorganisms causing blood stream infections to help the clinicians in making sound decisions when prescribing antibiotics to treat their patients.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.