ANTIMICROBIAL RESISTANCE TRENDS AMONG BLOODSTREAM PATHOGENS IN A TERTIARY CARE SETTING, PESHAWAR

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ABSTRACT

OBJECTIVES

This study aimed to identify common pathogens isolated from blood cultures and evaluate their antimicrobial resistance patterns.

METHODOLOGY

This retrospective observational study was conducted at Rehman Medical Institute, Peshawar, from January to December 2024. All positive blood cultures during the study period were processed using standard microbiological techniques and the Vitek 2.0 system. Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2024 guidelines. Organisms were categorized as per standard resistance classification. Data were analyzed in SPSS v27, with $p<0.05$ considered significant.

RESULTS

Among 569 blood cultures, the most common isolates were Staphylococcus aureus (27.9%), Salmonella (25%), and Klebsiella (13.8%). Organism distribution varied significantly with age ($p<0.001$). Most S. aureus were methicillin-resistant S. aureus (MRSA, 88.6%), with no vancomycin resistance. Enterococcus and Streptococcus showed minimal resistance except to erythromycin. Salmonella included 31% multidrug-resistant (MDR) and 55.6% extensively drug-resistant (XDR) strains, with XDR significantly more common in S. typhi, pediatric patients, and age groups ($p<0.001$, 0.001, 0.2). Highly resistant Klebsiella were frequent: extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) 83.3% and carbapenem-resistant Enterobacteriaceae (CRE) 62.8%, with ESBL significantly associated with age (p 0.04). In Escherichia coli, 69.4% were ESBL and 12.2% were CRE, with ward-specific variation in CRE ($p=0.002$). Serratia showed ESBL (66.6%) and CRE (50%), with CRE more frequent in males ($p=0.02$). About 84% of Acinetobacter strains were difficult-to-treat resistance (DTR) strains, significantly associated with intensive care unit (ICU) patients and middle-aged individuals ($p<0.03$, 0.04). Pseudomonas showed lower resistance, with a 23.5% DTR, strongly associated with ICU ($p=0.006$).

CONCLUSION

Highly resistant pathogens were found in BSI, particularly MRSA, XDR Salmonella, and ESBL/CRE-producing Klebsiella and Serratia. Significant associations with patient age and hospital location underscore the urgent need for continuous surveillance, strengthened infection control, and robust antimicrobial stewardship programs.

KEYWORDS: *Bloodstream Infection, Antimicrobial Resistance, Staphylococcus, Salmonella*

INTRODUCTION

Antimicrobial resistance (AMR) is a growing global health threat and accounts for 9% of all global deaths. In 2021, 1.14 million deaths were attributable to AMR, a figure estimated to reach 1.91 million by 2050. South Asia and Latin America/Caribbean have the highest all-age AMR mortality rates.[1] In Asia, 70% of antibiotic resistance is rising, making it a regional and global hazard.[2] The burden is higher in bacteremia, for which blood culture remains the gold standard for pathogen identification and AMR profiling.[1,2] Bacterial pathogens from blood cultures are evolving, with multidrug-resistant strains complicating empirical treatment.[1,2] Gram-negative bacteria, including carbapenem-resistant strains, show increasing

resistance. At the same time, Gram-positive organisms also demonstrate rising resistance to key antibiotics.[1,2,3,4,5] Resistance patterns vary by region.[1] Patient demographics, comorbidities, antibiotic use, invasive procedures, and hospital settings (ICUs, specialty units, general wards) all contribute to bacteremia risk and emergence of MDR organisms. Overuse and misuse of antibiotics further accelerate AMR.[1] Early, adequate antimicrobial therapy is crucial for better outcomes, with first-line antibiotic choices dependent on local epidemiology and resistance trends.[3] Continuous regional surveillance is essential to guide empirical treatment. Our study identifies pathogens isolated from blood cultures and analyzes their AMR patterns to strengthen evidence-based stewardship, improve infection control, and enhance patient care as AMR escalates.[6] To identify the common pathogen isolated on blood cultures and describe their antibiotic resistance pattern.

METHODOLOGY

This retrospective observational study was conducted at Rehman Medical Institute, Peshawar. Ethical approval was obtained from RMI-REC; informed consent for data use was taken at admission, with confidentiality maintained. All positive blood cultures from Jan 1, 2024, to Dec 31, 2024, were included. As per hospital protocol, 10-20 ml of blood was collected aseptically to avoid contamination with skin flora. Samples were inoculated into adult or pediatric blood culture bottles and incubated in the BacT/ALERT 3D system for 7 days. Positive bottles were subcultured onto Blood agar (BAP) and MacConkey agar (MAC), and a direct Gram stain was performed. Plates were incubated aerobically at 35 ± 2 °C for 16-18 h, and growth was examined. For Gram-positive cocci (GPC), a catalase test was performed; catalase-positive isolates were differentiated by tube coagulase and DNase (for S. aureus), while catalase-negative isolates were identified to the species level using Vitek 2.0. For Gram-negative rods (GNR), catalase, oxidase, and motility tests were performed, followed by API 10S, API 20E/20NE, and Vitek 2.0 for final identification. Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion on Mueller-Hinton agar (MHA). Zone diameters were interpreted using CLSI M100 Ed.33, CLSI M100 Ed.34:2024, and EUCAST v14 (2024). Colistin susceptibility was assessed on colistin screen agar (CLSI guidelines), and tigecycline by EUCAST 2020. Strains were categorized as susceptible or resistant to antibiotic groups. Gram-negative organisms were stratified into Enterobacterales (E. coli, Klebsiella spp., Serratia spp.) and non-Enterobacterales (Pseudomonas aeruginosa, Acinetobacter spp., Salmonella spp.). Resistance classifications were applied as follows: *MDR (Salmonella):* resistant to 1st-line (ampicillin, chloramphenicol) and 2nd line (fluoroquinolones). *XDR (Salmonella):* resistant to all three lines, sensitive only to meropenem or azithromycin. *ESBL (Enterobacterales):* resistant to 3rd-generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone), *CRE (Enterobacterales)*: resistant to ≥1 carbapenem (imipenem, meropenem, doripenem, ertapenem). *DTR (Pseudomonas/Acinetobacter):* resistant to all standard first-line, high-efficacy agents (all β-lactams, incl. carbapenems/β-lactamase inhibitors, and fluoroquinolones), but not necessarily to all drug classes.[7] All positive blood cultures from any ward during the study period were included. Cultures with Candida or contaminants (skin commensals such as coagulase-negative staphylococci, Corynebacterium spp., etc.) were excluded. Data were recorded on a predefined pro forma and analyzed using SPSS version 24. Descriptive statistics included frequencies and percentages for categorical variables (e.g., gender, ward, isolated organisms, and resistance pattern) and mean ± SD for continuous variables (e.g., age). The chi-square test was used to evaluate associations between categorical variables and resistance strains; when expected cell counts were low, Fisher's exact test was used. Age differences among resistance categories were assessed using an ANOVA. Statistical significance was defined as $p < 0.05$.

RESULTS

Table 1: Patient's Demographic and Common Pathogens

Patient's Demographics		%(n)
Gender	Male	58.5%(n=335)
	Female	41.5%(n=234)
Age groups	00-18	44.1%(n=251)
	19-60	36.6%(n=208)
	Above 60	19.3%(n=110)
Common Pathogens	Staphylococcus Aureus	27.90%(n=159)
	Streptococcus	3.50%(n=20)
	Enterococcus	3.30%(n=19)
	E coli	8.60%(n=49)
	Serratia	3.20%(n=18)
	Klebsiella	13.70%(n=78)
	Salmonella	25% (142)
	Acinetobacter	7.70%(n=44)
	Pseudomonas	03%(n=17)
	Others	04%(n=23)

Table 2: Antibiotic Resistance % of Pathogens Grown in Blood Cultures

Antibiotics	EC n=49	KP n=78	SA n=142	SE n=18	AC n=44	PS n=17	SAU n=159	ST n=20	EN n=19
AMK	6.2	51.3	-	61	79.5	23.5	-	-	-
AMC	93.6	86.3	-	-	-	-	-	-	-
AMP	96.5	-	76.8	-	-	-	94.2	10	63.2
SAM	-	-	-	-	94.1	-	-	-	-
AZM	-	-	0.7	-	-	-	-	-	-
CLOX	-	-	-	-	-	-	88.7	-	-
CLI	-	-	-	-	-	-	38.2	30.7	-
CFS	16.3	65.7	-	60	89.4	50	-	-	-
FEP	68	-	-	64.2	94.2	37.5	-	-	-
CTX	-	88	-	-	-	-	-	-	-
CAZ	75	47.4	-	57.1	92.8	31.7	-	-	-
CRO	76.5	82.8	57.7	64	94.8	NA	-	-	-
CZA	-	12.5	-	37.5	-	1.8	-	-	-
CIP	82.9	75.6	84.5	66.7	87.5	28	74	66.7	50
CST	-	1.78	-	-	00	-	-	-	-
ERY	-	-	-	-	-	-	-	81.25	85.7
DOX	11.1	25.5	-	-	0.26	-	17.7	0.2	0
GEN	30.4	53.5	-	-	76.1	-	-	-	-
LZD	-	-	-	-	-	-	0.06	-	-
MEM	12.2	62.8	4.9	50	83.7	26.6	-	00	27.2
MIN	5.8	-	-	-	NA	-	-	-	-
TZP	26	66.2	-	56.2	82	25	-	-	-
RIF	-	-	-	-	-	-	9.09	-	-
TGC	0.0	-	-	00	00	-	-	-	00
VAN	-	-	-	-	-	-	00	-	-

Note: EC = Escherichia coli; KP = Klebsiella spp.; SA = Salmonella spp.; SE = Serratia spp.; AC = Acinetobacter spp.; PS = Pseudomonas spp.; SAU = Staphylococcus aureus; ST = Streptococcus spp.; EN = Enterococcus spp.; AMK = Amikacin; AMC = Amoxicillin-Clavulanic acid; AMP = Ampicillin; SAM = Ampicillin-Sulbactam; AZM = Azithromycin; CLOX = Cloxacillin; CLI = Clindamycin; CFS = Cefoperazone-Sulbactam; FEP = Cefepime; CTX = Cefotaxime; CAZ = Ceftazidime; CRO = Ceftriaxone; CZA = Ceftazidime-Avibactam; CIP = Ciprofloxacin; CST = Colistin; ERY = Erythromycin; DOX = Doxycycline; GEN = Gentamicin; LZD = Linezolid; MEM = Meropenem; MIN = Minocycline; TZP = Piperacillin-Tazobactam; RIF = Rifampicin; TGC = Tigecycline; VAN = Vancomycin. - = sensitivity not checked.

Table 3(a): Resistant Pathogens and Their Association with Patients' Demographics

Demographics		Serratia n(%)				Klebsiella n(%)				E coli n (%)			
		ESBL 12(66.6)	P-Value	CRE 09(50)	P-Value	ESBL 65(83.3)	P-Value	CRE 49(62.8)	P-Value	ESBL 34(69.4)	P-Value	CRE 6(12.2)	P-Value
Gender	Male	10(71.4)	0.42	09(64.3)	0.02	44(84.6)	0.66	35(67.3)	0.24	14(70)	0.93	03(15)	0.62
	Female	02(50)		00		21(8.80)		14(53.8)		20(69)		03(10.3)	
Wards	Medicine and Allied	01(100)	0.70	01(100)	0.41	14(82.4)	0.20	10(58.8)	0.08	18(66.7)	0.40	02(7.4)	0.002
	Surgery and allied	00		00		05(100)		4(80)		03(75)		03(75)	
	Pediatrics	07(63.6)		06(54.5)		20(74.1)		14(51.9)		02(40)		00	
	ICU	04(66.7)		02(33.3)		25(92.6)		21(77.8)		07(87.5)		02(7.4)	
	OPD	00		00		01(50)		00		04(80)		00	
Mean Age mean ± SD		23.58±21.63	0.51	16.78±14.58	0.36	35.51±27.19	0.04	33.1±25.44	0.9	60.03±21.76	0.11	53±25.84	0.73

Table 3(b): Resistant pathogens and their association with patients' demographics

Demographics		Salmonella n(%)				Acinetobacter n (%)		Pseudomonas n (%)	
		MDR 44(31)	P value	XDR 70(55.6)	P value	DTR 37(84)	P value	DTR 4(23.5)	P value
Gender	Male	23(26.1)	0.11	54(61.4)	0.07	19(82.6)	0.77	01(11.1)	0.20
	Female	21(38.9)		25 (46.3)		18(85.7)		03(37.5)	
Wards	Medicine and Allied	29(38.7)	0.07	31(41.3)	0.001	02(66.7)	0.03	00	0.006
	Surgery and allied	00		00		00		00	
	Pediatrics	14(21.5)		47(72.3)		10(66.7)		00	
	ICU	01(50)		01(50)		25(96.2)		04(80%)	
	OPD	00				00			
Mean Age mean ± SD		21.11±14.93	0.07	16.1±12.23	0.02	39.19±26.65	0.04	51±10.86	0.33

DISCUSSION

In our study, Staphylococcus was the most prevalent organism, followed by Salmonella and Klebsiella. The predominance of Staphylococcus aligns with studies from Europe, Africa, and China, which consistently report Gram-positive bacteria as the leading cause of BSI.[8,9,10,11] In many studies, about half of positive blood cultures yielded Staphylococcus (≈50% or more), whereas our study showed a relatively lower prevalence.[8,10] Salmonella was the second most common isolate, consistent with studies from Pakistan (37%) and Africa (10.3%).[11,12] However, our percentage is considerably higher than reports from Kashmir (4.3%) and India (2%).[10,14] Literature from Western countries rarely identifies Salmonella as a primary isolate.[8,9,15,16] Klebsiella accounted for 13.5% of isolates, matching findings from Jordan (13.5%)[8] and comparable to China, where K. pneumoniae was isolated in 17.5% (639/3660) of cases.[15] This suggests its role as a significant Gram-negative pathogen globally. We identified 18 cases of Serratia in 1 year, a relatively higher frequency than reports of 45 cases over 8 years and 141 cases over 15 years (0.3/1000 prevalence).[17,18] This suggests a rising burden of Serratia BSI in our region. Pseudomonas was less common, consistent with other studies.[9,10,15] Antimicrobial resistance patterns further underscore the severity of the public health challenge. The most common isolate, Staphylococcus, demonstrated high resistance to penicillins and cephalosporins, similar to findings from Vietnam.[19] While data from Vietnam reported good sensitivity to quinolones, our study showed a higher resistance rate. Notably, 88.7% of Staphylococcus isolates in our study were MRSA, exceeding the 78.3% MRSA prevalence documented in another study from the same region.[20] Resistance to linezolid was remarkably low (0.06%) in our study, in contrast to 2.41% and 5.8% in other studies.[29,30] Importantly, none of our isolates were resistant to vancomycin, echoing global findings.[20,21,22] Our study revealed that Salmonella showed minimum resistance to Meropenem and Azithromycin. Ampicillin, Ceftriaxone, and Ciprofloxacin were highly resistant antibiotics. A similar study conducted in Karachi, Pakistan, in 2022 observed that ampicillin showed 98.38% resistance, followed by ciprofloxacin (87.09%) and ceftriaxone (51.61%). Meropenem also showed 3.22% resistance against Salmonella.[23] This study finding is similar to our study. Another study from Pakistan, conducted in 2024, reported that Salmonella typhi showed 0% resistance to both Meropenem and Azithromycin.[24] Moreover, the resistance against ampicillin was 31.6%, ceftriaxone and ciprofloxacin both showed 7.0% resistance.[24] This resistance is quite lower than what we have reported in our study, highlighting the more resistant Salmonella in our setup. In a European study, 90% of Salmonella isolates were resistant to ciprofloxacin, but only 28% were resistant to ampicillin, and only 4% were resistant to ceftriaxone.[25] Except for Ciprofloxacin, the rest of the drugs showed strikingly low levels of resistance in our study. In a 4-year study carried out in Taiwan, Salmonella showed 83.8% resistance to ampicillin, 22.7% to 3rd-generation cephalosporins, 3% to ciprofloxacin, and 3.3% to azithromycin. None of the strains was carbapenem-resistant.[26] Alarmingly, a few Salmonella isolates in our study exhibited resistance to meropenem and azithromycin-antibiotics often regarded as last-resort treatments. Such resistance is rarely reported and represents a significant threat to effective typhoid treatment.[24,25,26,27,28] Our study adds to the limited but growing body of evidence documenting carbapenem-resistant Salmonella, emphasizing the urgency of antimicrobial stewardship.[24,29,30] Likewise, we have also observed azithromycin resistance; it has been documented in only a few other studies.[27,28] Multidrug-resistant (MDR) and extensively drug-resistant (XDR) typhoid were detected in 28.8% and 57.7% of Salmonella cases, respectively. These figures closely mirror findings from Lahore (MDR: 21.9%, XDR: 56.8%), and exceed those reported in Peshawar in the recent past (MDR: 8.1%, XDR: 37.3%).[28,29] This trend indicates a growing public health burden of XDR typhoid, particularly in pediatric populations, as corroborated by other regional studies.[23,27,29] In other regions of the world, Octavia S et al. studied typhoid strains isolated from the Taiwanese population over 4 years and documented that 47.3% were MDR. 26 A study conducted over 4 years in England showed that 27% of typhoid was MDR and only 4% was XDR.[25] Despite this difference in prevalence of two resistant strains of Salmonella in different studies, the literature still speaks for a rising trend of overall resistant typhoid, regardless of which strain it is. In Europe and other parts of the world, resistant strains are also appearing; they, too, are among the travelers/visitors to Southeast Asia and Africa. [25,26] In our study, E. coli showed strikingly high penicillin resistance compared to other national and international studies.[8,9,12,14,28] Our study documented a very high (83.3%) prevalence of ESBL E. coli compared to studies from other countries, i.e., Gaza (51.6%) and Tanzania (31.13%).[32] Our study showed that 11.1% strains were CRE, which is similar to a study from Islamabad (13.1%) but higher than data from China (0.2%), Italy(0%), and Turkey(6.7%).[8,12,15,32] These findings highlight the high prevalence of carbapenem-resistant *E. coli (CRE)* strains in our setting. In our study, Klebsiella showed a large number of ESBL-E strains, consistent with

previous studies.[12,15,33] However, the prevalence of CRE strains was significantly higher than in China (62.8% vs 2.4%).[15] Moreover, resistance to aminoglycosides was notably higher in our study (53.5% to gentamicin) than in other studies worldwide.[9,10,12] *Serratia* in our study exhibited high-level resistance, including ESBL and CRE, in contrast to international studies, where resistance was generally low.[17,33] This suggests a potentially evolving resistance pattern in our region, warranting further surveillance. Pseudomonas isolates were less resistant overall than other Gram-negative organisms, consistent with findings from India.[10] However, resistance levels exceeded those reported in China and Istanbul.[9,15] Our study and other studies worldwide have shown a rising trend in DTR Acinetobacter, consistent with other studies; a few have also reported resistance to colistin and tigecycline.[9,10,15,30,34] However, in our study, 0% resistance was observed to colistin and tigecycline. Given regional variability and the dynamic nature of resistance, continuous surveillance is essential to guide empirical therapy, support antibiotic stewardship, and strengthen infection control measures. Such efforts are critical to mitigate the impact of AMR, preserve last-line antibiotics, and improve patient outcomes in bacteremia, where delays in effective treatment are associated with high mortality.

LIMITATIONS

Our study highlights the burden of AMR in bloodstream infections and identifies variations across age groups and hospital areas that can inform regional antibiograms and stewardship programs. A key strength is the use of the Difficult-to-Treat Resistance (DTR) framework, which has direct clinical applicability and is underexplored in regional literature. However, limitations include its retrospective design, which has limited causal inference. It is a single-centered study, which limits its generalizability. Inability to determine the sources of bacteremia and the lack of outcome data related to resistant strains are other limitations of this study. Exclusion of CoNS in this study may have underestimated the burden of bacteremia.

CONCLUSIONS

This study demonstrates that bloodstream infections in our setting are primarily driven by multidrug- and extensively drug-resistant organisms, with MRSA, XDR Salmonella, and ESBL/CRE-producing Klebsiella posing the most significant threats. Staphylococcus, especially MRSA, was the most frequent isolate, while the exceptionally high burden of MDR and XDR Salmonella, including rare resistance to meropenem and azithromycin, exceeds regional and global reports and is particularly alarming in pediatric populations. A relatively high number of DTR Serratia cases was also noted. Overall, resistance to penicillins, cephalosporins, quinolones, and aminoglycosides was high, with an emerging rise in carbapenem resistance. While colistin resistance remains rare, its regional presence highlights the need for vigilance.

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The authors accept responsibility for all aspects of the work and will ensure that any concerns regarding the accuracy or integrity of any part are properly investigated and resolved.