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Research Article

A Ten-Year Retrospective Analysis of the changing Antibiogram Pattern of Blood Isolates

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A B S T R A C T

Background: Bloodstream infections (BSIs) pose significant clinical challenges due to drug-resistant organisms, increasing morbidity, mortality, and healthcare costs.

Aim: This retrospective study analyses antimicrobial resistance (AMR) patterns in pathogens isolated from BSIs at Chettinad Hospital and Research Institute, over a decade, from 2013 to 2023.

Methods: Paired blood samples were inoculated into BacT/Alert media bottles and incubated in a Bactec automated blood culture system. Positive samples were cultured on blood agar and MacConkey agar plates, and pathogens were identified by their colony morphology and biochemical reactions. The Kirby-Bauer disc diffusion method was used to test how sensitive the bacteria are to antibiotics, following guidelines from the Clinical and Laboratory Standards Institute (CLSI).

Results: Findings show higher Gram-negative bacilli prevalence, peaking in 2023, with higher infection rates in paediatric patients and males. ICUs were primary BSI sites. Teicoplanin and vancomycin were CoNS (SosA)istently effective, while penicillin's efficacy fluctuated. Sensitivity to cotrimoxazole and cefepime has increased over time.

Conclusion: These results highlight the critical need for continuous surveillance and robust infection control measures to combat AMR and improve BSI treatment outcomes. Enhanced antimicrobial stewardship programmes are crucial for highlighting the increasing threat of drug-resistant pathogens.

Keywords: Bloodstream Infections, Antimicrobial Resistance, Intensive Care Units, Gram-Negative Bacilli

Introduction

Blood cultures play a vital role as specimens in diagnosing bloodstream infections (BSIs) and significantly impact the administration of proper antimicrobial treatment.[1] Drug-resistant organisms complicate BSIs, contributing to higher morbidity, mortality, and hospital expenses. The clinical and epidemiological CoNS (SosA)equences of antimicrobial resistance (AMR) present a concerning trend, imposing significant limitations and challenges for clinicians and healthcare policymakers.[2] The mortality rate in intensive care units (ICUs) resulting from BSIs can be exceptionally high, reaching up to 70%. The "Surviving Sepsis Campaign" advocates administering a suitable antimicrobial agent within 1 hour of suspecting sepsis, however, empirical therapy may prove ineffective in approximately one-fifth of cases.[3] In the epidemiological triad of BSI, determining the role of the infecting organism is frequently the most intricate aspect. Rigorous identification, relying on diverse parameters, is crucial for distinguishing between commensal flora and pathogenic microorganisms.[4] Even with the emergence of innovative testing methods, blood culture continues to be the primary method for diagnosing bloodstream infections, serving as the foundation for accurate identification.[5] The increasing incidence of fungemia in BSIs further exacerbates this challenging scenario.[3] In a hospital setting, AMR among pathogens is increasing. CoNS (SosA)tant surveillance is necessary due to variations in the local trends and epidemiology of BSIs. This study provides insights into antimicrobial resistance in pathogens from BSIs over the past decade, aiming to identify resistance trends and improve treatment and infection control.

Materials and Methods

Study Design

This retrospective study reviews the blood culture records of patients at Chettinad Hospital. This is a tertiary care teaching hospital, located in Kelambakkam, serving a diverse population, including rural, semi-urban, and urban areas, from January 2013 to December 2023. (a time period of 11 years). The approval for this study was obtained from the Institutional Human Ethics Committee of Chettinad Academy of Research and Education (IHEC-II/0506/24). As it was a retrospective study, spanning over a decade, the patient CoNS (SosA)ent was not obtained, however, the patient data was kept anonymised, to protect the patient privacy.

Sample Processing and Identification

Paired blood samples had been collected aseptically and inoculated into conventional Brain Heart Infusion broth bottles or Beckton Dickinson aerobic blood culture bottles, using adult or paediatric bottles as needed. Samples were incubated for 7 days in conventional bottles and 5 days in the Becton Dickinson BACTEC 9050 automated blood culture system, as shown in Figure 1. Positively flagged samples were sub-cultured on blood agar, chocolate agar, and MacConkey agar, while the conventional Brain Heart Infusion Broth (BHIB) bottles underwent sub-cultures on days 1, 3, and 6. Samples showing growth were processed for identification and antibiotic susceptibility, utilising colony morphology, gram staining, and biochemical reactions for pathogen identification.

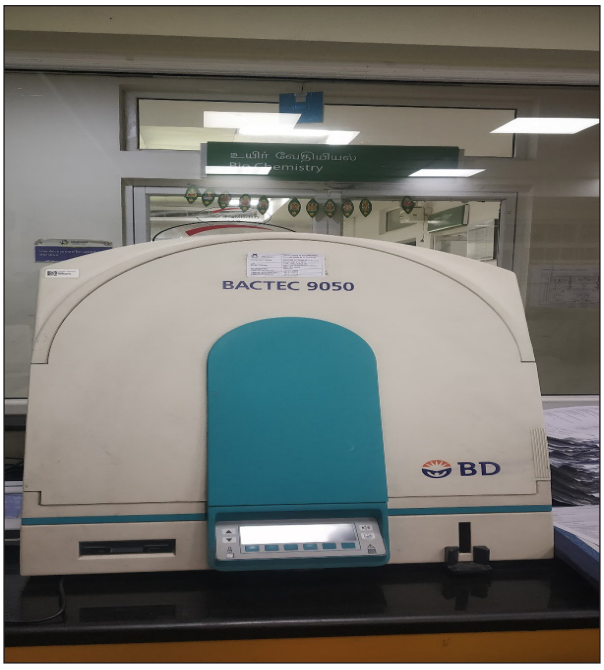

Figure 1.Becton Dickinson (BD)/ BACTEC 9050

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing, conducted annually under the updated Clinical and Laboratory Standards Institute (CLSI) protocols. Testing occurred across sample collection years, from 2013 to 2023.

Gram-positive organisms' susceptibility was evaluated using the following antibiotics: Ampicillin (AMP) (10µg), cefazolin (CZ) (30µg), chloramphenicol (C) (10µg), cloxacillin, clindamycin (CL) (10µg), ciprofloxacin (CIP) (5µg), cotrimoxazole (COT) (1.25/23.75µg), erythromycin (E) (15µg), gentamicin (GEN) (10µg), linezolid (LZ) (30µg), penicillin (P) (10µg), teicoplanin (TEI) (30µg), tetracycline (TE) (30µg), and vancomycin (VAN) (30µg).

Antibiotic susceptibility testing for Gram-negative organisms encompassed the following antibiotics: ampicillin (AMP) (10µg), amikacin (AK) (30µg), cefazolin (CZ) (30µg), cefepime (CPM) (30µg), cefotaxime (CTX) (30µg), ceftazidime (CAZ) (30µg), cefuroxime (CXM) (30µg), ciprofloxacin (5µg), cotrimoxazole (COT) (1.25/23.75µg), fosfomycin (FO)

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(200µg), gentamicin (10/25µg), imipenem (IMI) (10/25µg), meropenem (MER) (20/10µg), nitrofurantoin (NIT) (300µg), norfloxacin (NOR) (10µg), piperacillin-tazobactam (PIT) (100/10µg), polymyxin B (PB) (10µg), and tobramycin (TOB) (10µg).

Data collection and interpretation

The collected data from laboratory-confirmed BSI reports, both automated and manual records, were analysed and subsequently systematised by their year of isolation, pathogens, and antibiotic susceptibility profile. In reports, if a patient experienced two or more BSI incidents within 30 days following the initial isolation, each BSI occurrence was regarded as a new infection.

Statistical Analysis

Statistical analysis was conducted utilising MS Excel (Microsoft) spreadsheets to organise the collected data. Continuous variables were depicted as mean values (accompanied by standard deviation), while categorical data were presented as numbers or percentages. SPSS Statistics version 20.0 was employed for the statistical computations.

Results

Over the past decade, 2,625 samples yielded growth of bacterial pathogens. Figure 2 illustrates the year-wise distribution of isolates. Among them, 1419 samples yielded GPCs, the highest number was recorded in 2023, comprising 19% of the total, followed by 2019 at 11%. Similarly, out of 1,205 GNB isolated, the peak was also observed in 2023, CoNS (SosA)tituting 17% of the total, with 2018 following closely at 13%. Table 1 illustrates the age and gender distribution of these cases. Most isolates were from males, with 1,582 cases (60%), compared to 1,043 cases (40%) from females. The most bacterial isolates were from patients aged 0-10 years (37%), followed by those older than 60 (16%). The male-to-female ratio was 1.52:1, and the mean age of the patients was 30.26 ± 23.62 years.

Over the past decade, CoNS (SosA) prevalence increased from 52% in 2013 to 86% in 2023, with peaks in 2018 and 2021. S. aureus fluctuated, peaking at 22% in 2016 but dropping to 7% in 2023. *Enterococcus spp.* remained low, peaking at 12% in 2013 and 2017 and dipping to 2% in 2016 and 2023. These trends, shown in Figure 3, illustrate varying bacterial isolates.

Over the past decade, *E. coli* prevalence among GNB remained stable, fluctuating between 22% and 29%, with a dip to 11% in 2019. *Klebsiella spp.* increased from 11% in 2013 to 26% in 2023, with significant rises in 2017 and 2018. *S. typhi* showed wide variations, from 36% in 2013 to a low of 4% in 2021, then peaking at 47% in 2023, as shown in Figure 4.

Age and sex distribution of bloodstream infections from 2013 to 2023, detailing the number of GPC and GNB isolates per age group, was shown in table 1. For example, in the 0-10 age range, there were 610 GPC and 356 GNB isolates, totalling 966 cases.

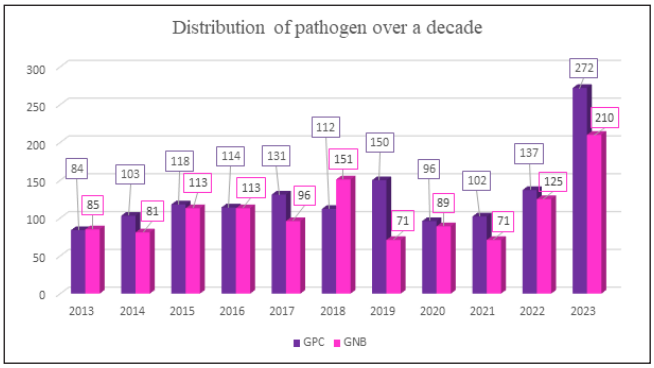

Figure 2.Distribution of organisms causing BSI based on year-wise isolation from 2013 to 2023.

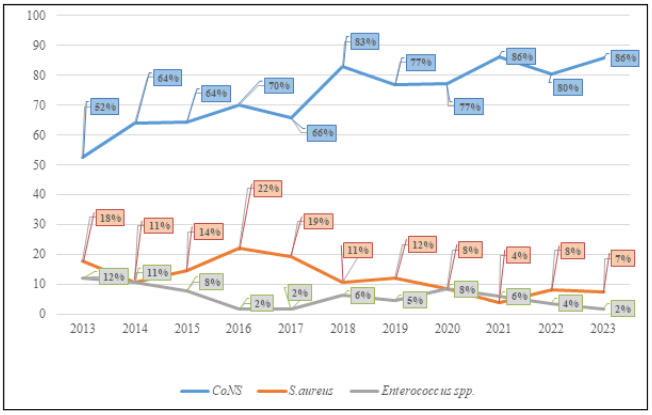

Figure 3.Illustrates the prevalence trends of commonly isolated GPC over the last decade, highlighting significant changes in their distribution and frequency

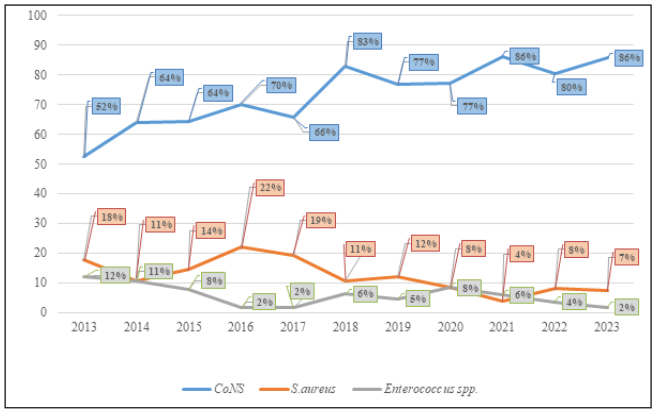

Figure 4.Illustrates the prevalence trends of commonly isolated GNB over the past decade, emphasizing notable changes in their distribution and frequency

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Table 1.Analysis of age and sex-wise distribution of organisms causing Bloodstream Infection from 2013 to 2023

	Categories	GPC	GNB	Total
Age distribution	0-10	610	356	966
	11-20	86	95	181
	21-30	113	163	276
	31-40	94	80	174
	41-50	132	120	252
	51-60	168	180	348
	>60	210	218	428
Gender	Male	834	748	1582
	Female	579	464	1043

Table 2.Analysis of location-wise distribution of organisms causing Bloodstream Infection from 2013 to 2023

	Ward/OP	GPC	GNB
Location	NICU and PICU	174	113
	SICU and GICU	215	280
	Private ward	103	90
	Pediatric ward	218	114
	Medicine wards	198	212
	ETC ward	25	18
	Outpatients	480	385
	Total	1413	121

Table 2 presents the distribution of BSI across hospital wards and outpatient settings, highlighting GPC and GNB isolates. NICU/PICU had 174 GPC and 113 GNB isolates, SICU/GICU had 215 GPC and 280 GNB, and outpatients had 480 GPC and 385 GNB isolates. These findings inform targeted healthcare interventions.

Table 3 presents data on the sensitivity of GPCs in blood samples over the past decade, including *CoNS (SosA), S.aureus*, and *Enterococcus spp*. Vancomycin was CoNS (SosA) istently effective against all strains. Tetracycline showed efficacy against *S. aureus* and *Enterococcus spp.*, while Teicoplanin was effective against *CoNS (SosA)* and *S. aureus*. These findings highlight the most effective antibiotics for treating bloodstream infections caused by these GPCs.

Table 4 details the antibiotic sensitivity of GNBs isolated from blood samples over ten years, focusing on *E.coli, Klebsiella* spp., and *S.typhi. E.coli* accounted for 20% of isolates, and *Klebsiella spp.* 17%, and *S.typhi* 16%. AMP was effective against *S.typhi* (178 sensitive observations) but less so against *E.coli* (28) and *Klebsiella spp.* (2). CIP showed higher activity against *Klebsiella spp.* (149 sensitive isolates). These insights are crucial for tailoring treatment strategies for BSI caused by GNBs.

Over the past decade, the sensitivity of *S.aureus* to cotrimoxazole increased from 47% in 2013 to 95% in 2023, highlighting enhanced antibiotic effectiveness. Similarly, *Acinetobacter spp.* susceptibility to cefepime rose from 57% to 90% during the same period, indicating improved efficacy against infections. These trends, depicted in Figure 5, underscore significant improvements in antibiotic sensitivity over time.

Table 3.Commonly isolated GPCs from the blood samples over 10 years

	Antibiotics	*CoNS (SosA)* n=1065 (75%)	*S. aureus* n=166 (12%)	*Enterococcus spp.* n=74 (5%)
Number of sensitive isolates	Ampicillin	30	1	36
	Cefazolin	290	62	IR
	Chloramphenicol	105	39	NR
	Ciprofloxacin	729	65	11
	Clindamycin	703	101	NR
	Cloxacillin	501	99	NR
	Cotrimoxazole	608	81	NR
	Erythromycin	363	83	46
	Gentamycin	898	97	56
	Linezolid	877	117	62
	Penicillin	244	15	68
	Teicoplanin	861	133	58
	Tetracycline	652	139	67
	Vancomycin	872	133	63

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Table 4.Commonly isolated GNBs from the blood samples over 10 years

	Antibiotics	*Escherichia coli* n = 243 (20%)	*Klebsiella spp.* n= 206 (17%)	*Salmonella typhi* n = 196 (16%)
Number of sensitive isolates observed	Amikacin	202	141	NR
	Ampicillin	28	2	178
	Cefazolin	48	25	NR
	Cefepime	79	142	NR
	Cefotaxime	59	39	NR
	Ceftazidime	0	85	NR
	Cefuroxime	51	32	NR
	Ciprofloxacin	56	149	27
	Cotrimoxazole	89	62	182
	Gentamycin	136	127	NR
	Imipenem	203	134	NR
	Meropenem	196	137	NR
	Piperacillin Tazobactam	186	168	NR
	Tobramycin	21	132	NR
	Tetracycline	-	-	180
	Chloramphenicol	-	-	27

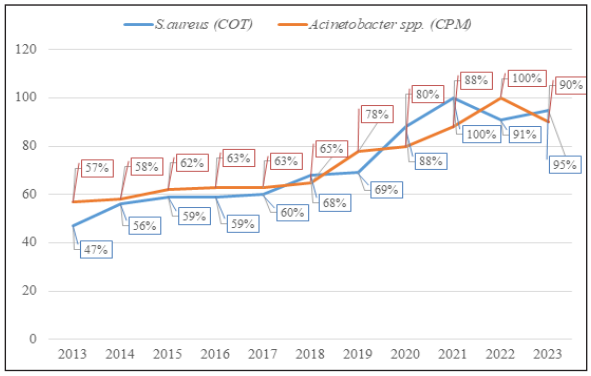

Figure 5.Increasing efficacy of drugs against blood isolates the over past decade

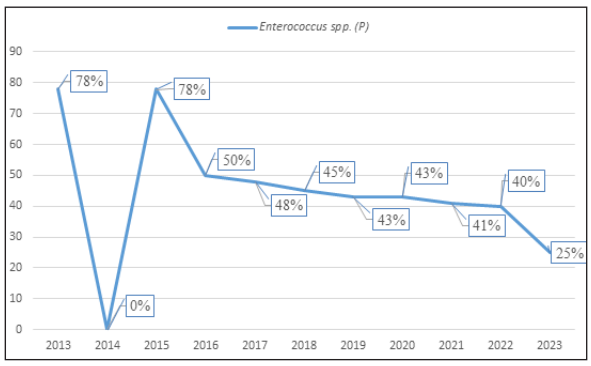

Figure 6.Decreasing efficacy of penicillin against blood isolates of Enterococci, over the past decade

The susceptibility of *Enterococcus spp.* to penicillin has shown a declining trend over the past decade. Sensitivity dropped sharply from 78% in 2013 to 0% in 2014, briefly recovered to 78% in 2015, but then continued to decline. By 2023, sensitivity had fallen to 25%, reflecting ongoing challenges in managing Enterococcus infections and the need for alternative treatments. Figure 6 illustrates this decrease in penicillin sensitivity against *Enterococcus spp.*

Discussion

Our study provides comprehensive insights into the AMR patterns of BSIs over the past decade. By analysing blood culture records from Chettinad Hospital and Research Institute, we identified notable trends in the distribution of pathogens, age and gender demographics, and antibiotic susceptibility profiles among isolated organisms.

The rise in BSIs during the study period underscores the growing burden on healthcare systems. GNB were more commonly isolated than GPC, with varying prevalence over time, CoNS (SosA)istent with Alonso-Menchén D. 2024 findings.[6] The significant increase in BSI cases, especially in 2023, highlights the urgent need for proactive prevention and management strategies.

Our findings reveal distinct age and gender distributions among BSI cases, with a higher prevalence observed in males and paediatric patients aged 0-10 years. This

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observation is CoNS (SosA) istent with the findings reported by Garrido MM in 2019.[7] These demographic trends reflect the vulnerability of specific populations to bloodstream infections and underscore the importance of targeted interventions and surveillance strategies to mitigate risk factors and improve outcomes.

Location-wise analysis of BSIs revealed that ICUs are hotspots for BSI acquisition, followed by medicine and paediatric wards. This contrasts with Yangzom T's findings, which identified medicine wards as the most common location for bacterial isolates, followed by ICUs.[8] These results highlight the importance of infection control practices, such as hand hygiene, environmental cleaning, and antimicrobial stewardship, in minimising pathogen transmission in high-risk settings.

Antibiotic susceptibility testing provided valuable insights into the efficacy of commonly used antimicrobial agents against isolated pathogens. Our data demonstrate CoNS (SosA)istent activity of Teicoplanin and vancomycin against prevalent GPCs, including *CoNS (SosA)*, *Staphylococcus aureus*, and *Enterococcus spp*, these same findings were reported by Narayanan S in 2020 and Ohata K in 2020.[9,10] Similarly, amikacin exhibited high sensitivity rates against *E.coli* and *Klebsiella spp.* In contrast, AK El-Sayed reported a 79% resistance to amikacin.[11] while imipenem and meropenem showed significant efficacy against *E.coli*, a statement corroborated by B. Mohsenpour in 2024.[12]

Of particular concern are the fluctuating susceptibility rates observed for certain antibiotics, such as penicillin against *Enterococcus spp.*, indicating evolving resistance patterns and challenges in antimicrobial therapy, the same statement was given by L Herrera-Hidalgo[13]. The observed increase in the efficacy of Cotrimoxazole and Cefepime against *S.aureus* and *Acinetobacter spp.* reflects the dynamic nature of antibiotic resistance and the potential impact of targeted interventions on improving treatment outcomes. These same findings were reported by L Li in 2024 and SS Atakov in 2023.[14,15]

In our study, we observed that the prevalence of *CoNS (SosA)* has increased over the past few decades, a trend similarly noted by Giulia Grassia in Italy in 2024.[16] We also observed that *S. aureus* and *Enterococcus spp.* were the second most frequently isolated Gram-positive bacteria in blood samples, with their prevalence estimated below 25%. This finding is CoNS (SosA)istent with statements by Olivia Sochi Egbule et al. in Nigeria and Samiha AIDAOUI in Algeria, both in 2024.[17,18]

In our findings, we observed a prevalence of *S. typhi* at 47% in 2023, compared to 36% in previous reports. In contrast, T. Roberts et al. (2024) reported a prevalence of 72.7%, while Philip M. Ashton et al. (2024) reported a prevalence of 30% in blood samples.[19,20] *E. coli* and *Klebsiella spp.* were also found in blood samples, with prevalence estimates ranging from 11% to 29% over the last decade. Similar prevalence rates were observed and reported by KB Laupland in 2008 and Abd El Tawab AA et al. in 2015.[21,22]

Conclusion

This past decade has seen a notable rise in BSIs, especially among males and paediatric patients. ICUs are critical sites for BSI acquisition, emphasising the need for strong infection control measures. While antibiotics like teicoplanin and vancomycin remain effective against GPC, the fluctuating efficacy of penicillin against *Enterococcus spp.* highlights ongoing resistance challenges. Encouragingly, the improved effectiveness of Cotrimoxazole and Cefepime demonstrates successful antimicrobial interventions, underscoring the importance of continuous AMR surveillance.

Conflict of Interest: None

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Author's Contribution: The study was conceptualized by AMK and PS- Methodology, formal analysis, and investigation were carried out by AMK and PS. The original draft of the manuscript was prepared by AMK- Review and editing were conducted by APS and PS. Overall supervision was provided by PS.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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