Annals of Clinical Microbiology and Antimicrobials

RESEARCH

Open Access

Major blood stream infection-causing bacterial pathogens, antimicrobial resistance patterns and trends: a multisite retrospective study in Asmara, Eritrea (2014–2022)

Yosan Gebremeskel Andemichael[1], Eyorusalem Tsehaye Habtetsion[1], Hagos Hayelom Gulbet[1], Maedn Hailemariam Eman[2], Oliver Okoth Achila[3], Samuel Tekle Mengistu[4], Azania Werede Andemichael[5], Abrehet Marikos Buthuamlak[2], Eyob Yohannes Garoy[3], Berhe Tesfai[6] and Mohammed Elfatih Hamida[7*]

Abstract

Background An important knowledge gap exists on the epidemiology of blood stream infections (BSIs) in low-middle-income countries (LMICs). In this retrospective analysis, we evaluated the etiology, antimicrobial resistance (AMR) and trends of BSIs in Eritrea.

Methods The study reviewed 9-year records (January 2014– December 2022) of 3153 patients with blood culture results available in the National Health Laboratory (NHL) archives. Relevant data included age, sex, hospital/care center, and year.

Result During the surveillance period, we examined data from 3153 patients (1797 (57.0%) men vs. 1356 (43.0%) females, and 1.2 years (Q1: 0.01 months - Q3: 15 years). Of the samples submitted, 1026 (35.5%) samples were positive for the presence of pathogens (663(64.6%) pathogens vs. 363 (35.4%)) potential contaminants. In decreasing frequency, the most common isolates were: Coagulase-negative *Staphylococcus* (CoNs), 189 (28.6%); *Klebsiella spp.*, 120 (18.2%); *Escherichia coli*, 66 (10.0%); *Citrobacter spp*., 48 (7.3%); *Staphylococcus aureus*, 47(7.1%); *Pseudomonas aeruginosa*, 34 (5.1%); and *Salmonella spp.*, 33(5.1). The relative prevalence of BSIs changed somewhat over time (p-value < 0.001) with the isolation of multiple isolates trending upward from 2018 and onwards. Additional findings included the likely presence of extended spectrum beta lactamase (ESBL), high frequency of methicillin resistant *Staphylococcus aureus* (MRSA) (37(80.4%) and high rate of resistance to gentamicin (363(62.5%) and fluoroquinolones. Furthermore, the multiple antimicrobial resistances (MAR) index was relatively high (mean = 0.55, SD: ±0.23) with wide species-level variation. In a related density cluster analysis, we demonstrated a time-dependent increase in the diversity of resistotypes.

Conclusion This study highlights the considerable health burden of AMR/or MDR in BSIs in Eritrea. Additionally, it underscores the urgent need for enhanced laboratory capacity, surveillance, institutionalisation of antibiotic

*Correspondence:
Mohammed Elfatih Hamida
mohelfatih77@gmail.com

Full list of author information is available at the end of the article

stewardship programs, and robust infection control programs in hospitals across the country. The need for multidisciplinary research was also highlighted.

Keywords Blood stream infections, Sepsis, Bacteraemia, Multidrug resistance, MRSA, Eritrea, And Africa

Introduction

Blood stream infections (BSIs) have been described as a leading cause of death worldwide [1]. According to a recent Global Burden of Disease (GBD), collaborators report, the annual incidence of BSIs ranges between 80 and 257 per 100,000 person years (PYs) [2]. In a related GBD collaborators report, estimates suggested that there were 48.9 million (95% uncertainty interval [UI] 38.9 million– 62.9 million) incident cases of sepsis worldwide in 2017 and 11.0 million (UI: 10.1 million– 12.0 million) deaths [1]. This accounted for 19.7% (18.2–21.4) of all global deaths. Other epidemiological indexes such as pathogen-specific incidence, age-standardized mortality rate; years of life lost (YLL); disability-adjusted life-years (DALY) also suggest a growing problem across all GBD super-regions [2].

The high burden of death and morbidity associated with antimicrobial resistance (AMR) has been linked to a number of factors including variations in the frequency / burden of BSIs across GBD super-regions [3, 4]. In particular, several reports have established the presence of several pathogens in the WHO critical- and high-priority pathogens list in LMICs (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species* (ESKAPE) organisms in particular [5, 6]. The presence of these pathogens, these authors conceded; is compounded by a high prevalence of AMR and / or multidrug resistance (MDR).

Although a severe dearth of comparative studies on outcomes associated with antimicrobial susceptible organisms and AMR/or MDR exists; a recent meta-analytical review demonstrated that AMR-related infections were generally associated with worse outcomes including higher crude mortality (Odds ratio (OR) 1.58 [95% confidence interval (CI) 1.35–1.80]), higher odds of admission to intensive care units (OR 1.96 [95% CI: 1.56–2.47]); excess direct medical costs (US Dollars $12 442 [95% CI: US Dollars $6 693– US Dollars $18 191]) and greater length of hospital stay (LOS) [7]. Other investigators have reported similar results [8–10] with some studies suggesting that the problem is largely driven by specific drug-pathogen combinations. These include methicillin resistant *Staphylococcus aureus (MRSA);* vancomycin resistant *Enterococcus* spp.; carbapenem-resistant *Enterobacteriaceae*; 3rd generation cephalosporin resistant *Enterobacteriaceae* (3GC-R), among others [11–13].

Globally, there is a realisation that the threat associated with AMR in BSIs is at a crisis point. At the same time, the problem is still understudied and is poorly documented in LMICs [4, 14]. This outcome is largely driven by under-resourced laboratories, inadequate manpower, lack of adequate diagnostics, and lack of surveillance networks [15]. In 2015, the WHO-led Global Antimicrobial and Surveillance System (GLASS) was established to help address this data gap. Although the number of countries participating in the WHO-GLASS-AMR program has increased; several gaps remain [4]. Key gaps include poor data quality due to low testing coverage, technical and methodological gaps in surveillance modalities; limited use of locally relevant data in policy formulation; and the absence of data from some countries.

In Eritrea, for example, data on BSIs AMR is extremely limited. This problem is highlighted by the fact that the country has not submitted data to successive GLASS-AMR program data calls [4]. The paucity of data from the country is driven by multiple challenges. These include an extremely limited clinical microbiology laboratory (CML) coverage; overreliance on empirical treatment; gaps in the management of medical information– lack of integration of laboratory and clinical data; and gaps in the training of laboratory personnel. As a consequence, information on AMR in BSIs in the country depends heavily on modelling estimates [1].

In the past, experts have emphasized the need for locally relevant data on AMR. Underscoring this position, they have argued that without AMR data, it is extremely difficult to make rational recommendations or monitor the effectiveness of in-country interventions. For a country like Eritrea, low quality data may translate into poor empiric antibiotic treatment strategies, soaring AMR rates, inability to track and map the spread of AMR, detect early outbreaks, monitor intervention efforts, undertake informed prioritization of resource allocation, and set data-driven AMR intervention strategies and standards. Due to the absence of data on AMR in BSIs in Eritrea; this study aimed to identify common etiological agents for BSIs from several tertiary level hospitals with a large volume of patients in Asmara, Eritrea, and evaluate AMR and MDR patterns and trends.

Methods

Study setting, patient population, and design

This study sought to identify blood stream infection-causing bacterial pathogens, their antimicrobial susceptibility patterns, and associated trends in Eritrea, using secondary information collected from pediatric and adult patients. Overall, the study used 9-year (January

2014– December 2022) worth of data from the National Health Laboratory (NHL) logbook. The use of data at the NHL was premised on a number of considerations. First, the institution serves as the reference medical laboratory for all health institutions in Eritrea. More importantly, it is the only institution in Eritrea with a microbiology unit that can culture bacteria or perform antimicrobial sensitivity tests (AST).

In the period under study (2014–2024), 2342 samples were submitted by the Orotta Pediatric Hospital (OPH); 747 by Orotta National Reference Hospital (ONRH). Haz Haz, Sembel and others submitted 25, 14 and 25 samples respectively. Figure 1 shows the distribution of positive samples. Further, it must be emphasised that some of the patients from the stated national reference hospitals were referred from other care centres in the country. This means that patients from these institutions are from different regions and come from a wide pool of speciality clinics / wards including medical, intensive care units (ICU), oncological, surgical, outpatient departments, urological, gynecological, general and subspecialized surgical units, among others.

Data collection methods

The study reviewed 9 years (January 2014– December 2022) of archival records of blood culture results in 3153 patients. Data were collected from laboratory records by trained laboratory personnel and subsequently entered into Excel spread sheets. After individual parsing of the captured data, deduplication was performed using the deduplication functionality of the R statistics software R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Culture and identification of bacteria

An evaluation of the protocol for blood cultures used at the NHL did not uncover any changes over the study duration. The protocol requires the use of 5 ml and 2 ml of blood from adults and children, respectively. The collected blood samples were inoculated onto brain heart infusion bottles in 1:10 ratio and incubated at 37 ° C for 18–24 h. After incubation, the samples were sub-cultured onto Chocolate agar. Negative blood culture results were followed for a minimum of 5 days. Macroscopic colony and Gram stain characteristics were used for preliminary evaluation and presumptive identification of Gram-negative (Gram– ve) and Gram positive (Gram + ve) isolates was undertaken through a series of biochemical tests. Isolates were subsequently subjected to antimicrobial susceptibility testing (AST). The shipment of samples and the evaluation of bacterial contaminants were based on in-house guidelines.

Antimicrobial Susceptibility Testing (AST)

AMR testing was performed using the disk diffusion method (modified Kirby–Bauer method) on Mueller-Hinton agar. Antibiotics used for Gram-negative bacteria included: amikacin (30 µg); gentamicin (10 µg), cotrimoxazole/trimethoprim– sulphur, methoxazole (TMP-SMZ) (1.25/23.75 µg), cephalexin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), vancomycin (30 µg), clindamycin (2 µg), penicillin G (10 units), oxacillin (4 ug / ml), ampicillin (10 µg), erythromycin (15 µg), nitrofurantoin (300 µg). ATCC culture *Staphylococcus aureus* ATCC 25,923 and *Escherichia coli* ATCC 25,218 are normally used as controls.

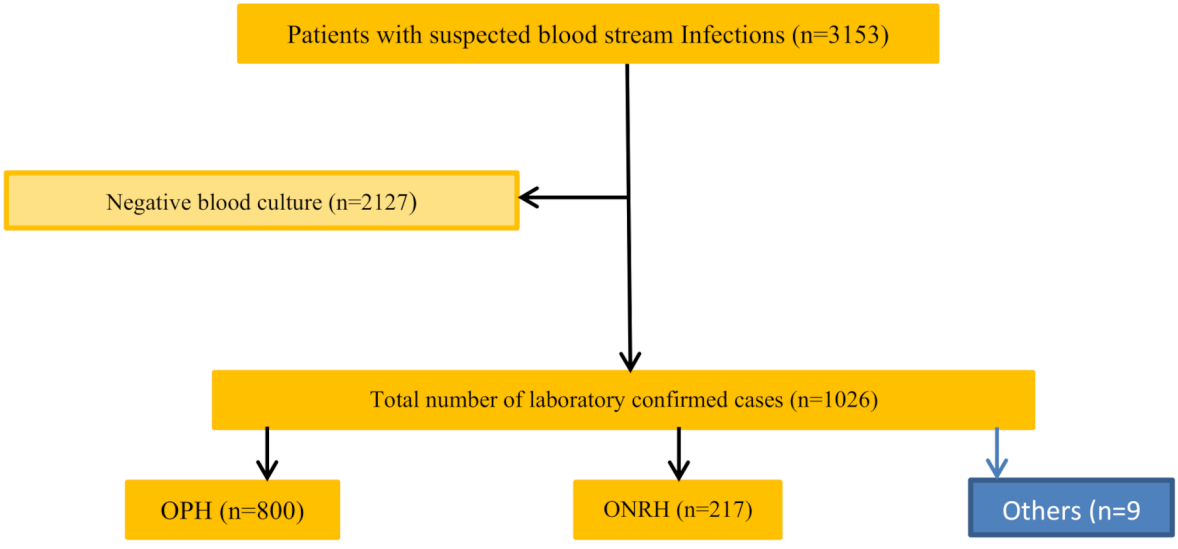


Fig. 1 Flow chart of patient data

Finally, the minimal inhibition concentration (MIC) breakpoints of the Clinical and Laboratory Standards Institute (CLSI) are used to define AMR [16]. In this analysis, characterisation of antibiotic susceptibility was based on the latest CLSI guidelines [16]. According to these specifications, isolates are categorised as "S" = Susceptible; Intermediate = 'I' and Resistant 'R. In our analysis, I and R were merged (I + R) and were subsequently reported as 'R'.

Determination of MDR and Multiple Antibiotic Resistance Index (MAR) and identification of contaminants

According to the European Center for Disease Prevention and Control and other agencies; MDR resistance can be defined as the in vitro nonsusceptibility to one or more drugs/agents in three or more classes of antibiotics [17]. Multiple antibiotic resistance (MAR) index was evaluated using the formula: MAR = n (number of agents to which the isolates were resistant)/N (total number of agents tested) [18]. Blood culture contamination was calculated using the formula: (Isolates of Coagulase-negative staphylococci (CoNs), Gram positive bacilli (GPB), and *viridans* group of *Streptococcus* without AST data) / (Total number of recovered isolates) X 100.

Statistical analysis

Data collected from the NHL register was entered into an Excel Spreadsheet. For subsequent analysis, the information was transferred to the Statistical Package for Social Sciences (SPSS) version 23 software (IBM Co., Armonk, NY, USA) or R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). The Gaussian distribution of the data was tested using the Shapiro-Wilks test. Where appropriate, mean [± standard deviation (± SD)] or median [interquartile range (IQR)] and related parametric and nonparametric statistics (t-test and ANOVA or Mann Whitney U and Kruskal Wallis) were used. For categorical variables, Chi-square tests (χ^2) and /or Fishers exact test were used. Trends per year were evaluated using Cochran-Armitage test for trends. *P*-values < 0.05 were considered statistically significant.

Ethical considerations

This study relied exclusively on information on BSIs and associated metadata registered in the NHL logbook. To use this information, ethical approval was obtained from the Ministry of Health Research Ethics and Protocol review committee (approval number: Ref: 05/2022). To meet the specific consent condition set by the ethical committee, personal identifiable information was deleted during the preliminary phase of data collection. As consequence, informed consent was not required from patients.

Results

General description of patient data

During the surveillance period (January 2014– December 2022 = 9 years), data from 3153 patients (1797 (57.0%) men vs. 1356 (43.0%) females) from multiple institutions - mainly in Asmara, was retrieved. The median age (IQR) was 1.2 years (Q1: 0.01 months, Q3: 15 years). Importantly, significant year-to-year variation in testing was observed across disparate age scales. For example, we observed a decline of samples from neonates (< 28 days old) from 316 (46.8%) in 2016 to 0.0 in > 2020. In patients in the > 28 days – 1 year age range, an increase was observed from 100 (14.8%) to 322 (33.8%). Similar increases were observed for patients > 15 years of age. Table 1.

Blood culture positivity

Recovery rate was calculated from the 3153 samples submitted. Based on the previously stated formula, the recovery rate was 1026 (32.5%). Disaggregation of recovery rate with respect to year, location, age and sex demonstrated the following: that recovery rates differed across scalar years: < 2016 (161(22.9%); 2017–2018 (118 (27.0%)); 2019–2020 (312 (34.4%)), and > 2020 (435(33.8%); *p* value < 0.001. Recovery rates also differed with respect to location. In decreasing frequency, the rates were 800 (34.0%) for OPH; 217(29.0%) for ONRH; 3(21.4%) for Sembel; 3(12.0%) for Haz Haz and 3(12.0) for others (*p* value = 0.002). Rates of isolation also differed with respect to age. Figure 2.

Characterisation of pathogens

Of the 1026 recovered isolates, 663(64.6%) were identified as pathogens while 363 (35.4%) were identified as potential contaminants. In decreasing frequency, the proportions of pathogenic bacteria were as follows: CoNs, 189 (28.6%); *Klebsiella spp.*, 120 (18.2%); *Escherichia coli*, 66 (10.0%); *Citrobacter spp*., 48 (7.3%); *Staphylococcus aureus*, 47(7.1%); *Pseudomonas aeruginosa*, 34 (5.1%); and *Salmonella spp*., 33(5.1). In contrast, *Acinetobacter spp.*, and *Streptococcus pneumoniae* were isolated small in numbers: 5 (0.8%) and 2(0.3%).

The relative prevalence of BSIs changed somewhat with time (*p* value < 0.001). For example, a large proportion of *Enterobacter spp.*, and *Salmonella spp.*, BSI were isolated after 2020–17 (56.7%) and 26(78.8%), respectively. Similarly, the isolation of *Klebsiella spp*., *Escherichia coli*, and *Pseudomonas aeruginosa* trended upward in later years (after 2018).

Table 1 Year-to-year comparisons of patient demographic profiles, sample distribution, and pathogen distribution of blood stream infection data from the national microbiology laboratory, Asmara, Eritrea: 2014–2022

Characteristics	≤ 2016 *n* (%)	2017–2018 *n* (%)	2019–2020 *n* (%)	> 2020 *n* (%)	*p*-value (χ^2)	Total *n*(%)
Population	675 (21.4)	437 (13.9)	907(28.8)	1134(36.0)	-	3153
Gender						
Male	364(53.9)	248(56.8)	529(58.3)	656(57.8)	0.309 (3.6)	1797 (57.0)
Female	311(46.1)	189(43.2)	378(41.7)	478(42.2)		1356(43)
Age (years)						
<1 month	316(46.8)	103(23.6)	286(31.5)	0(0.0)	< 0.001 (594.17)	705(23.7)
>1 month– 1year	100 (14.8)	92(21.1)	149(16.4)	322(33.8)		663(22.3)
>1–15 years	165(24.4)	140(32.0)	237(26.1)	284(29.8)		826(27.8)
>15–40 years	66(9.8)	70(16.0)	113(12.5)	173(18.2)		422(14.2)
> 40 years	28(4.1)	32(7.3)	122(13.5)	173(18.2)		355(11.9)
Hospital						
OPH	579(85.8)	332(76.0)	668(73.6)	763(67.3)	< 0.001(86.9)	2342(74.3)
ONRH	82(12.1)	98(22.4)	230(25.4)	337(29.7)		747(23.7)
Others	14(2.1)	7(1.6)	9(1.0)	34(1.1)		64(2.0)
Gram Staining						
Gram Negative	44(36.7)	39(60.0)	113(63.1)	146(61.9)	< 0.001(25.5) [b]	342(57.0)
Gram Positive	76(63.3)	26(40.0)	66(36.9)	90(38.1)		258(43.0)
Culture outcome						
Positive cultures	161(23.9)	118 (27.0)	312 (34.4)	435(38.4)	< 0.001 (48.24) [b]	1026(32.5)
Positive						
Positive - Pathogens	124 (18.4)	70 (16.0)	186(20.5)	283(25.0)	< 0.001 (61.03)	663(64.6)
Positive - Potential contaminants	37(5.5)	48(11.0)	126 (13.9)	152(13.4)		363 (35.4)
Common isolates						
CoNs	60 (48.4)	15 (21.4)	49 (26.3)	65 (23.1)	< 0.001 (137.34)	189 (28.5)
Enterobacter spp.	3 (2.4)	3(4.3)	7(3.8)	17(6.0)		30 (4.5)
Staphylococcus aureus	10(8.1)	8(11.4)	14(7.5)	15(5.3)		47(7.1)
Klebsiella spp.	25(20.2)	15(21.4)	39(21.0)	41(14.6)		120(18.2)
Escherichia coli	7(5.6)	7(10.0)	23(12.4)	29(10.3)		66(10.0)
Pseudomonas spp.	2(1.6)	5(7.1)	18(9.7)	9(3.2)		34 (5.1)
Salmonella spp.	3(2.4)	0(0.0)	4(2.2)	26(9.3)		33 (5.0)
Citrobacter spp.	2(1.6)	6(8.6)	17(9.1)	23(8.2)		48(7.3)
Acinetobacter spp.	2 (1.6)	2(2.9)	1(0.5)	0(0.0)		5(0.8)
Streptococcus pneumoniae	1 (0.15)	1(0.15)	0 (0.0)	0(0.0)		2(0.3)
GPB	0(0.0)	0 (0.0)	0 (0.0)	35(12.5)		35 (5.3)
Other GNB	5 (4.0)	3(4.3)	10(5.4)	12(4.3)		30(4.5)

Note. Data are presented as mean standard deviation or n (%). Abbreviations: OPH: Orotta Pediatric Hospital; ONRH: Orotta National Referral Hospital; CoNs: Coagulase negative Staphylococcus; GPB: Gram positive bacilli: other Gram-negative Bacteria (GNB): *Proteus spp.*, *Providentia spp*, Haemophilus *influenza*. B: Cochran-Armitage trend test

Antimicrobial drug resistance in blood Stream isolates from specific clinics in Asmara, Eritrea

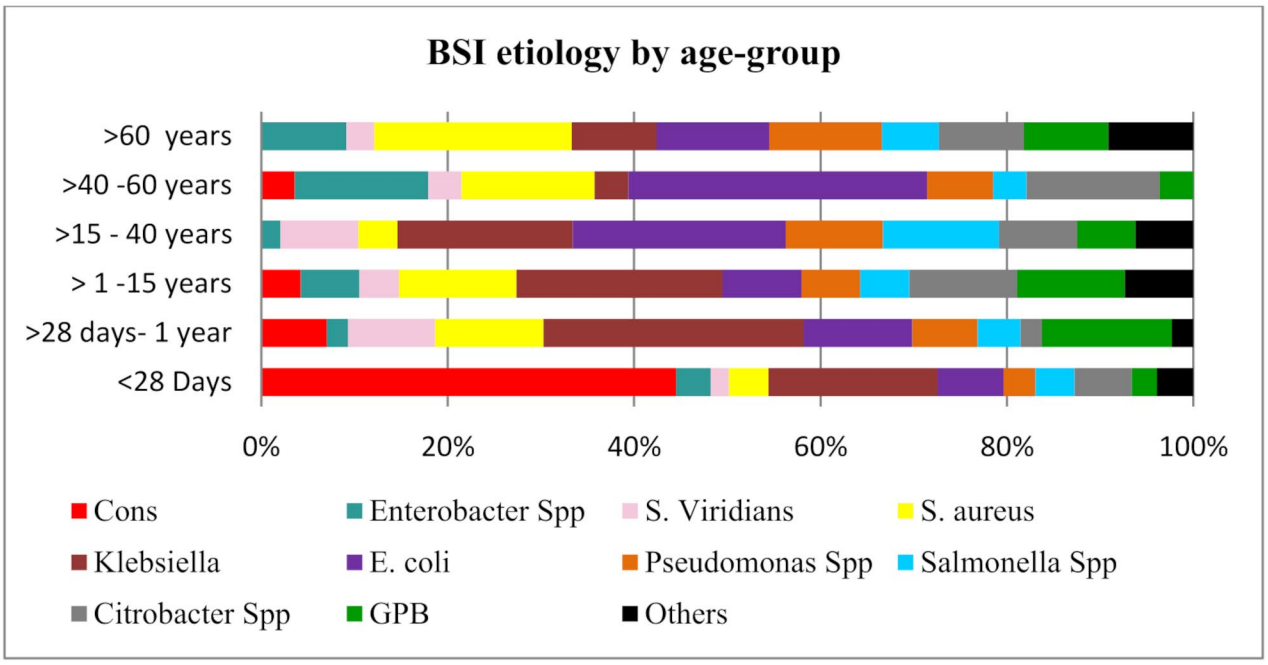
Resistance of gram-positive bacteria

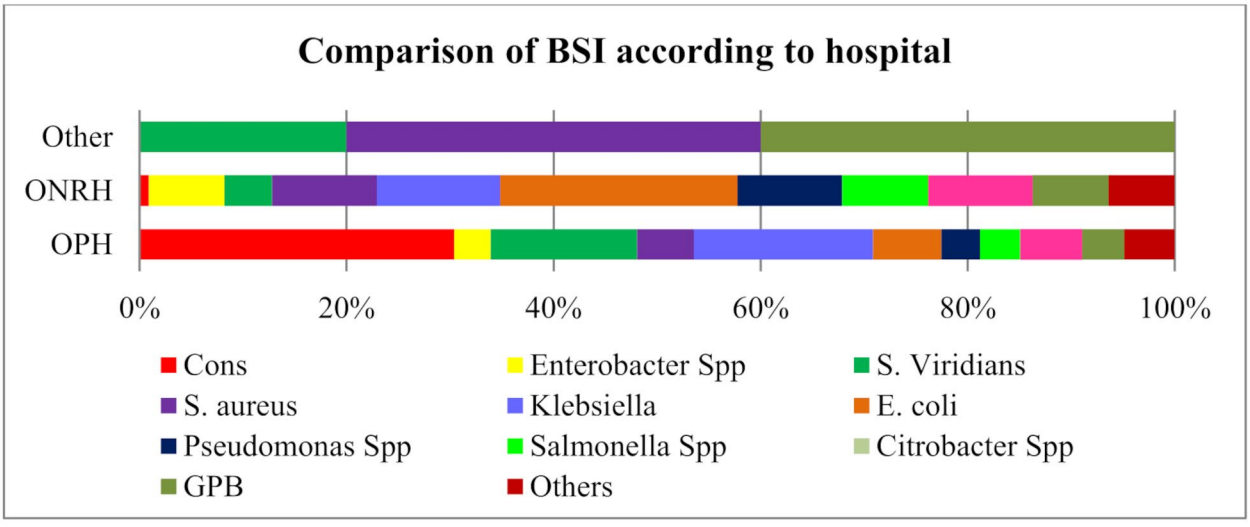
In the present study, the antimicrobial resistance profile of *Staphylococcus aureus and* CoNs was evaluated. AMR patterns are summarized in Table 2. Overall, 37(80.4%) of the *Staphylococcus aureus* isolates were resistant to Oxacillin (marker of MRSA). Resistance to gentamicin, TMP-SMZ, ciprofloxacin, tetracycline, erythromycin, penicillin, ampicillin and vancomycin stood at 104 (56.5%), 26 (68.4%); 19(45.2%), 18(43.9%), 18 (42.9%), (38 (86.4%), 37(80.4%), 13(27.2%) respectively.

Among CoNs, resistance to penicillin, ampicillin, Oxacillin, vancomycin, gentamicin, stood at 168 (92.3%), 171 (94.5%) and 171(94.5), 66(35.5%), and 104 (56.5%), respectively. Finally, a low resistance rates was observed for chloramphenicol, 23(12.8%).

Resistance of gram-negative bacteria

Escherichia coli had a relatively high rate of resistance to gentamicin, 36 (55.4%); TMP-SMZ, 261(71.7%); 3rd generation cephalosporins (3GC) - cephalexin, 44 (60.8%), ceftazidime, 39 (60.9%), ceftriaxone, 39 (60.9%); tetracycline, 41(66.1%); and ampicillin 61(92.4%). Moderately high resistance was observed for quinolones - nalidixic acid, 32(52.5%) and ciprofloxacin 30(46.9%); and chloramphenicol, 17(26.2%). Although the number of





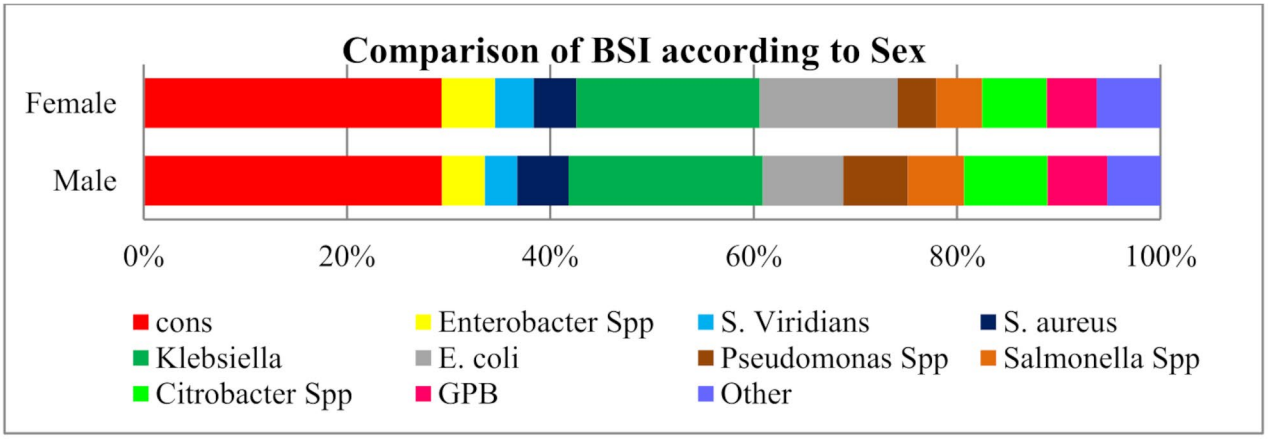


Fig. 2 Graphs showing the distribution of pathogens with age, sex, and hospital

Table 2 Antibacterial resistance profiles of bacteria and pathogens isolated from blood culture in patients from Eritrea, 2014–2022

Antibiotic	Overall *R* (%)[1]	E. coli *R* (%)	Klebsiella spp. *R* (%)	Entero-bacter *R* (%)	Pseudo-monas spp *R* (%)[1]	Citrobac-ter *R* (%)[1]	Salmo-nella spp *R* (%)	Other GNB *R* (%)[1]	S. aureus *R* (%)	CoNs *R* (%)
Aminoglycosides										
Amikacin	27(8.6)	7(11.9%)	5(4.9)	2(7.4)	5(15.6)	4(9.1)	1 (3.7)	3(11.1)	-	-
Gentamicin	363(62.5)	36(55.4)	97(83.7)	16(57.1)	18(54.5)	39(81.2)	17(54.8)	22(71.0)	14(31.1)	104(56.5)
Anti folate										
TMP-SMZ	261(71.7)	47(75.8)	77(74.8)	19(67.9)	18(54.5)	36(76.6)	20(69.0)	17(77.3)	26(68.4)	1(50)
Cephalosporins										
Cephalexin [3rd]	273(83.7)	44(69.8)	92(86.8)	25(89.3)	25(78.1)	45(95.7)	23(79.3)	-	-	-
Ceftazidime[3rd]	233(68.5)	39(60.9)	84(74.3)	14(48.3)	22(66.7)	36(76.6)	23(74.2)	15(65.2)	-	-
Ceftriaxone [3rd]	251(73.0)	39(60.9)	95(81.5)	22(75.9)	16(48.5)	40(85.1)	21(67.7)	18(78.3)	-	-
Quinolone										
Nalidixic acid [1st]	112(34.5)	32(52.5)	20(18.7)	7(25.9)	19(57.6)	19(40.4)	4(13.8)	9(47.4)	2(100.0)	
Ciprofloxacin [2nd]	115(29.4)	30(46.9)	21(18.8)	9(31.0)	8(25.0)	11(22.9)	2(6.5)	15(50.0)	19(45.2)	0(0)
Phenicols										
Chloramphenicol	188(32.6)	17(26.2)	46(40.7)	14(48.3)	20(62.5)	23(50)	18(58.1)	17(48.6)	10(21.7)	23(12.8)
Tetracyclines										
Tetracycline	213(57.0)	41(66.1)	50(48.1)	15(55.6)	23(71.9)	28(60.9)	19(65.5)	-	18(43.9)	1(50.0)
Glycopeptide										
Vancomycin	80(32.8)	-	-	-	-	-	-	-	13(27.7)	66(35.5)
Lincosamides										
Clindamycin	6(12.8)	-	-	-	-	-	-	-	6(14.3)	0.0)
Beta-Lactams										
Penicillin	206(90.7)	-	-	-	-	-	-	-	38(86.4)	168 (92.3)
Oxacillin	208(91.2)	-	-	-	-	-	-	-	37(80.4)	171(94.5)
Ampicillin	333(94.3)	61(92.4)	112(95.7)	29(96.7)	31(93.9)	45(93.8)	25(80.6)	30(83.3)	-	-
Macrolides										
Erythromycin	18(42.9)	-	-	-	-	-	-		18(42.9)	0
Other										
Nitrofurantoin	155(42.2)	12(19.0)		14(51.9)	25(80.6)	31(66.0)	4(13.8)	16(72.7)	4(9.8)	1(33.3)

Abbreviation: Overall R: Overall resistance; GNB– Gram-Negative Bacteria; GNP– Gram positive bacteria, CoNs: coagulase negative staphylococcus; TMP-SMZ: Trimethoprim sulfamethoxazole;

Description: Other GNB: Other gram-negative bacteria

Escherichia coli isolates tested for resistance to amikacin was low, it had the lowest resistance, 7(11.9%).

Among *Klebsiella pneumoniae*, high resistance rates were observed for gentamicin, 97(83.7%); TMP-SMZ 47(75.8%); 3GC, cephalexin, 92 (86.8%); ceftazidime, 84 (74.3%); ceftriaxone, 95(81.5%); and ampicillin, 112 (95.7%). In addition, a moderately high rate of resistance was observed for chloramphenicol, 46(40.7%) and tetracycline, 50(48.1%). On the contrary, lower resistance rates were observed for quinolones nalidixic acid (20 (18.7%) and ciprofloxacin, 21(18.8%) and amikacin, 5(4.9%).

In *Pseudomonas aeruginosa*, high resistance was observed for gentamicin; 3GC; chloramphenicol; tetracycline; and ampicillin. Among the relatively low number of isolates tested, a lower resistance to amikacin and ciprofloxacin was observed, 5 (15.6%) and 8 (25%).

Finally, *Salmonella Typhi* isolates exhibited a high rate of resistance for all agents tested except amikacin 1(3.7%) and quinolones (nalidixic acid, 4(13.8%) and ciprofloxacin, 2(6.5%). Resistance profiles for the other GNB are shown in Table 2.

Trends in antimicrobial drug resistance in blood stream infections in patients from Eritrea

Trends in AMR in BSIs were evaluated for specific microorganisms. Detailed resistance rates for selected antibiotics per year are shown in Table 3. For example, *Klebsiella spp*., *Escherichia coli*, *Pseudomonas aeruginosa*, CoNs and *Staphylococcus aureus*, exhibited high (> 91.3%) and sustained rates of resistance to ampicillin. Most notably, *Klebsiella spp.*, exhibited reduced resistance to several agents– ceftazidime, peak of 14 (93.3%) in 2017–2018 and 22(55.0) in > 2020, p value 0.005; ciprofloxacin, 4(26.7%) in 2017–2018 and 6(15%) in > 2020, p value < 0.001; TMP-SMZ, 10(83.3%) in < 2016 to 23(57.5%) in > 2020, p-value < 0.020; tetracycline 8(61.5%) in 2017–2018 to 16(40%) in > 2020, p value 0.002; and chloramphenicol, 16(66.7%) in < 2016 to 9(22.5%) in 2020, p value 0.003. On

Table 3 Resistance patterns for specific pathogens for selected antibiotics per year

Isolates	< 2016 *n*(%)	2017–2018 *n*(%)	2019–2020 *n*(%)	> 2020 (%)	*P* value (χ^2)[b]
Klebsiella spp.,					
Ampicillin	21 (91.3)	14(100)	37(100)	40(100)	0.234 (8.05)[b]
Ceftazidime	17(73.9)	13(92.9)	32(88.9)	22(55.0)	**0.015 (15.71)**[b]
Ceftriaxone	19(76.0)	14(93.3)	32(86.5)	30(75.0)	0.212 (8.38)
Ciprofloxacin	0 (0)	4(26.7)	11(29.7)	6(15.0)	**< 0.001 (31.9)**b
TMP-SMZ	10(83.3)	11(78.6)	33(89.2)	23(57.5)	0.020 (15.05)
Gentamicin	22(88)	14(93.3)	34(91.9)	27(69.2)	0.105 (10.51)
Tetracycline	1(6.2)	8(61.5)	25(71.4)	16(40.0)	**0.002 (21.25)b**
Chloramphenicol	16(66.7)	9(64.3)	12(34.3)	9(22.5)	**0.003(19.7)b**
Escherichia coli					
Ampicillin	5(83.3)	6(100)	23(100)	27(93.1)	0.444 (5.82)
Ceftazidime	3(50)	5(83.3)	17(73.9)	14(35.9)	0.325 (6.95)
Ceftriaxone	2 (33.3)	4(66.7)	14(60.9)	19(65.5)	0.480 (5.51)
Ciprofloxacin	1(20)	4(57.1)	9(39.1)	16(55.2)	0.384 (6.36)
TMP-SMZ	3(100)	6(85.7)	18(78.3)	20(69)	0.542(2.15)
Gentamicin	2(33.3)	4(57.1)	16(69.6)	14(48.3)	0.140 (9.65)
Tetracycline	1(25)	4(57.1)	17(73.9)	19(67.9)	0.25(7.84)
Chloramphenicol	2(28.6)	2(28.6)	8(36.4)	5(17.2)	0.489 (2.42)
Pseudomonas aeruginosa					
Ampicillin	2(100)	4(80.0)	16(94.1)	9(100)	0.351(6.69)
Ceftazidime	1(50)	4(80.0)	14(82.4)	3(33.3)	
Ceftriaxone	1(50)	4(80)	7(41.2)	4(44.4)	0.409 (6.13)
Ciprofloxacin	0 (0)	1(25.0)	4(23.5)	3(33.3)	0.928 (1.91)
TMP-SMZ	2(100)	2(40.0)	7(41.2)	7(77.8)	0.315 (7.06)
Gentamicin	1(50)	3(60)	8(47.1)	6(66.7)	0.946(1.69)
Tetracycline	0 (0)	3(60)	12(70.6)	8(88.9)	0.357(6.62)
Chloramphenicol	1(50)	3(60)	10(62.5)	6(66.7)	0.642(4.26)
CoNs					
Vancomycin	10(17.2)	1(6.7)	12(24.5)	43(65.2)	**< 0.001 (46.2)**
Oxacillin	54(90)	11(100)	48(98.0)	58(95.1)	0.361(6.59)
Gentamicin	23(38.3)	9(69.2)	47(25.5)	64(34.8)	**< 0.001(24.46)**
Chloramphenicol	3(5.5)	3(20)	7(14.3)	10 (16.7)	0.515 (5.23)
Staphylococcus aureus					
Oxacillin/MRSA	9(90)	7(87.5)	10(71.4)	11(78.6)	0.330(5.00)
Ciprofloxacin	0 (0)	4(57.1)	10(71.4)	5(33.3)	**0.045(12.87) b**
Vancomycin	2(20)	1(12.5)	3(21.4)	7(46.7)	6.97(0.324)
TMP-SMZ	3(100)	4(57.1)	10(71.4)	9(64.3)	0.58 (1.97)
Erythromycin	1(20)	6(75.0)	6(42.9)	5(33.3)	0.29(7.37)
Clindamycin	2(33.3)	0(0)	2(14.3)	2(13.3)	0.554(4.92)
Total					
Ampicillin	38(84.4)	38(97.4)	111(96.5)	146(94.8)	**0.012(16.29)**
Ceftazidime	28(63.6)	29(80.6)	97(87.4)	79(53.0)	**< 0.001(40.36) b**
Ceftriaxone	28(59.6)	31(86.1)	84(75.0)	108(72.5)	**0.039(13.3)**
Ciprofloxacin	3(6.7)	17(36.2)	49(38.3)	46(26.9)	**< 0.001(43.66)**
TMP-SMZ	19(76.0)	32(69.6)	100(78.7)	110(66.3)	0.062(12.00)
Gentamicin	56(47.9)	43(70.5)	127(73.8)	137(59.3)	**< 0.001(32.8)**
Tetracycline	5(15.2)	24(52.2)	85(67.5)	99(58.6)	**< 0.001(38.76)**
Chloramphenicol	28(24.8)	29(46.0)	63(36.8)	68(29.7)	**0.018(15.3)**

Abbreviation: CoNs: coagulase negative staphylococcus; TMP-SMZ: Trimethoprim sulfamethoxazole

Number of tested isolates/numbers of resistant isolates (% resistant). Superscript b: linear-to-linear association. Statistics

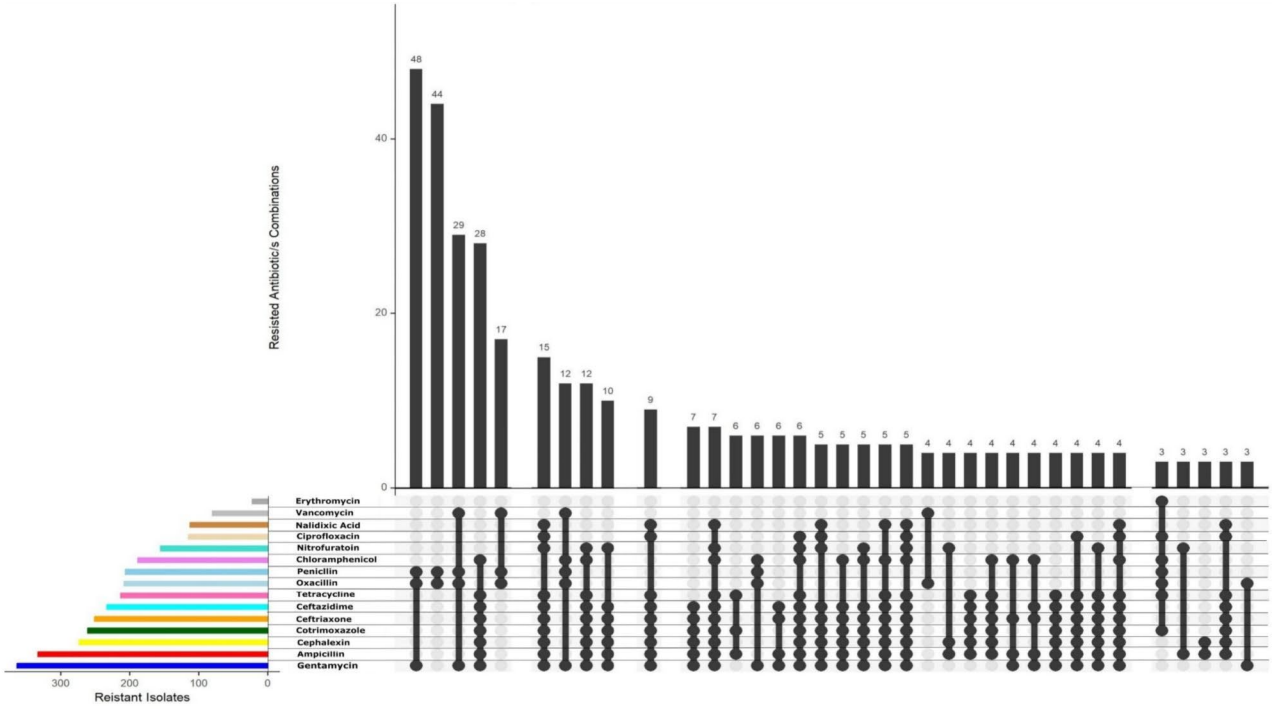


Fig. 3 Fifty most common resistotypes in isolates from patients with BSIs in Eritrea

Table 4 Prevalence of multidrug resistance (MDR) positivity among commonly isolated bacteria that cause sepsis in patients in Asmara, Eritrea: 2014–2021

Year	Multi-drug resistance in uropathogenic bacteria (MDR in BSIs)								
	E. coli*n* (%)[1]	Klebsiellaspp. *n* (%)[1]	Enterobacterspp. *n* (%)[1]	Pseudomonasspp. *n* (%)[1]	Citrobacter *n* (%)[1]	Other GNB *n*(%)[1]	Salmonella sppn (%)	Staphylococcus aureus*n* (%)	CoNs (%)
≤ 2016	3(42.9)	23(92.0)	3(100)	2(100)	1(100)	6(75)	2(66.7)	7(70.0)	25 (41.7)
2017–2018	6(85.7)	14(93.3)	2(66.7)	5(100)	6(100)	4(80)	-	6(75.0)	9(60.0)
2019–2020	21(89.7)	35(94.6)	7(100)	16(94.1)	16(94.1)	10(90.9)	3(100)	10(71.4)	35(71.4)
> 2020	26(89.7)	32(80.0)	16(94.1)	9(100)	22(95.7)	11(91.7)	19(76.0)	10(66.7)	50(76.9)
***P* value**	**0.012 (10.87)[b]**	0.174 (4.97)	0.245 (4.16)	0.808 (0.971)	0.069 (7.08)	0.681(1.50)	0.576(1.10)	0.980 (0.19)	**< 0.001 (18.67)** [b]
Total	56(84.8)	104(88.9)	28(93.3)	32(97.0)	45(93.8)	31(86.1)	24(77.4)	33(70.2)	119(63.0)

Abbreviations: GNB– Gram-Negative Bacteria; GPB - Gram-positive bacteria, Superscript b: Linear-to-linear association. Other GNB: Proteus spp, Providentia spp.

the contrary, *Staphylococcus aureus* exhibited reduced resistance to ciprofloxacin, from 4(57.1%) in 2017–2018 to 5(33.3) in > 2020, *p*-value 0.045.

Resistotypes in BSI–related pathogens

Analysis of 50 most frequent resistotypes was undertaken. In decreasing frequency, resistance to both gentamicin and ampicillin was as follows: 39(83.0) in *Citrobacter spp.*; 93(80.9%) in *Klebsiella pneumoniae;* 18(64.3%) in *Enterobacter spp.;* 37(57.8%) in *Escherichia coli; 17(54.8% in Salmonella spp.; 18(54.5%)* in *Pseudomonas aeruginosa.* Figure 3 presents a summary of 50 most common resistotypes.

Time series illustrating the prevalence and trends of multidrug resistance (MDR) in blood stream isolates from patients in Eritrea, 2014–2021

During the entire surveillance period, MDR was reported in 472 (79.1%) of the isolates. In *Escherichia coli*, MDR increased from a low point of 3(42.9%) in the period < 2016 to 26(89.7%) in > 2020. This translated into an overall MDR of 56(84.8%). In CoNs, overall MDR resistance was 119(63.0%). MDR increased from 25(41.7%) in the period ≤ 2016 to 50(76.9%) in > 2020 period. In other organisms, MDR rates were persistently high (*Klebsiella spp.*, 104 (88.9%); *Enterobacter spp.*, 28(93.3%); *Pseudomonas spp.*, 32(97.0%); *Citrobacter spp.*, 45 (93.8%); Other GNB, 31(86.1%); *Salmonella spp.*, 24(77.4%); *Staphylococcus aureus*, 33(70.2%)). Table 4.

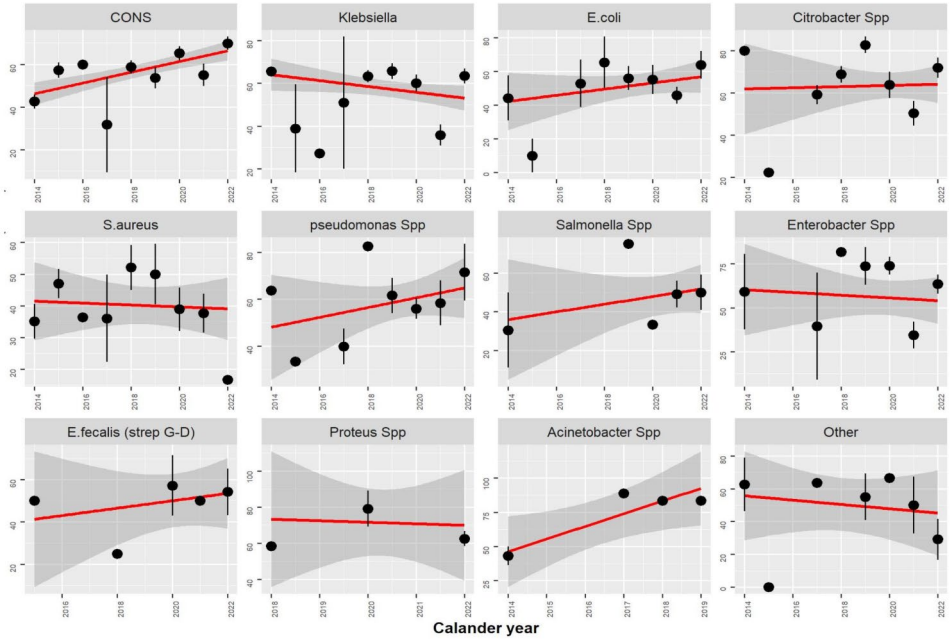


Fig. 4 Trends in MDR and related 95% CI for specific isolates

Table 5 Multidrug resistance index patterns of bacterial isolates from blood culture of patients from specific hospitals in Asmara, Eritrea, 2014–2022

Number of resistant isolates (*R*)	Bacterial isolates								
	E. coli *n*(%)	Klebsiella spp. *n* (%)	Enterobacter *n* (%)	Pseudomonas spp *n* (%)[1]	Citrobacter *n* (%)[1]	Salmonella spp *n* (%)	Other GNB *n* (%)[1]	S. aureus *n* (%)	CoNs *n*(%)
R0	2	0	1	0	0	3	**1**	**1**	5
R1	7	8	1	0	1	4	3	4	13
R2	1	5	0	1	2	0	1	9	52
R3	7	3	6	1	1	2	4	5	70
R4	7	11	2	4	3	2	5	4	37
R5	3	8	3	3	3	0	4	7	12
R6	2	14	1	7	6	1	4	7	-
R7	7	22	3	3	6	4	4	7	-
R8	9	24	4	3	6	12	3	1	-
R9	12	14	6	6	7	2	1	2	--
R10	8	8	2	2	10	0	4	0	-
R11	1	0	1	3	3	1	2	0	-
MAR index	**0.52**	**0.57**	**0.56**	**0.58**	**0.62**	**0.49**	**0.55**	**0.4**	**0.58**

Abbreviations: E. coli: Escherichia coli; GNB: Gram Negative bacteria; CoNs: Coagulase negative staphylococcus; n: Number of isolates; R: resistance, R0: No resistance; R1: Resistant to 1antibiotic; R2: Resistant to 2 antibiotics

Separately, analysis of trends demonstrated an increase in MDR for CoNs and *Acinetobacter* spp., p-value < 0.001 and 0.04, respectively. In contrast, *Klebsiella spp.*, demonstrated a reduction in MDR (p value < 0.05). Other isolates had relatively high, albeit stable changes, in MDR trends. Figure 4.

Multidrug resistance index patterns in blood stream isolates from Asmara, Eritrea

The analyses of the MAR index demonstrated that 472 (79.02%) of the isolates had a MAR index > 2. Among gram–ve isolates, 13 (2.01%) were fully susceptible (MAR = 0) and 11 (1.84%) were resistant to all agents tested (MAR = 1). Table 5. Furthermore, analysis of trends demonstrated a significant cluster of resistotypes in later years. Supplementary Fig. 2 and Fig. 5.

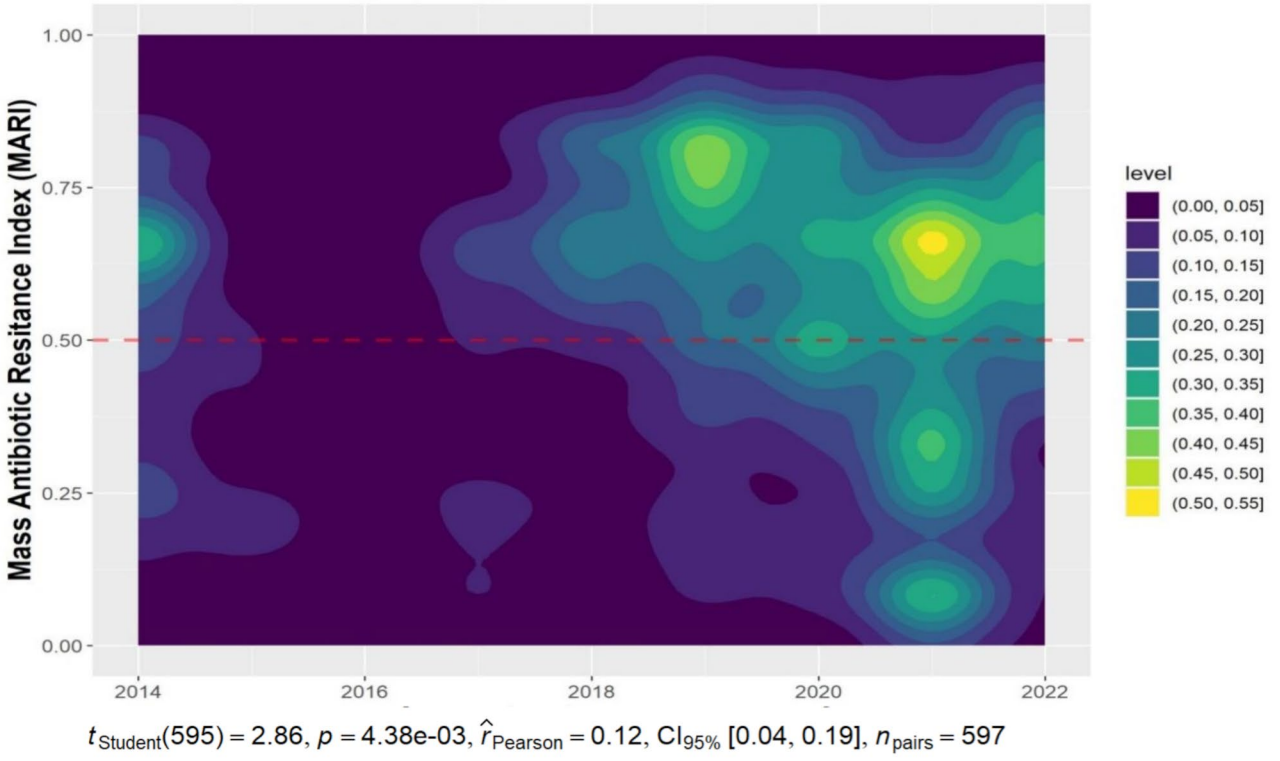


Fig. 5 Variation from year to year in the clustering of total MAR in patients with BSIs in Eritrea. Data from National Health Laboratory, 2014–2022

Discussion

In their 2022 report, the GLASS-AMR group of experts noted that a high percentage of AMR and AST tests within a country are essential to optimise the empirical treatment of BSIs. Unfortunately, there is a wide intra- and inter-country/or regional variation in testing coverage. In a recent analysis of LMIC vs. HIC data on BCIs, the researchers demonstrated that HICs tend to have greater testing coverage (> 229.4–826.6 per 1 000 000 vs. < 0.7–3.3 per 1000 000 population for countries in East Africa [4]. In our study, we observed that over a 9-year period, the NHL received ~ 3153 blood culture samples from a population of ~ 4 000 000 or 87.58 samples per 1 000 000 population per year.

Under-utilisation of clinical microbiology services (CMS), even in the treatment of patients with sepsis; is a well-documented phenomenon in LMICs [4, 19]. The severity of this problem can be demonstrated by the large gap between sepsis estimates in LMICs and BCI per 1000 000 population. For most countries in East Africa, for example, sepsis estimates are below the recent GBD BCI per 1000 000 population estimates: > 2500 to < 3400 per 100 000 vs. < 0.7–3.3 per 1000 000 [1, 4]. All in all, one inescapable conclusion from these results is that the CMS in NHL is underutilised and that the treatment of sepsis in Eritrea is largely unguided by AST data.

The limited range of antibiotics tested and the limited range of media used in this laboratory can undermine diagnostic and surveillance functions. Examples of antibiotics that were not tested but are widely used in the country included amoxicillin [20]. Other agents included Amoxicillin/clavulanic acid (Augmentin©) – used in the combination disk diffusion test (CDDT) for ESBL -/AmpC β-lactamases (AmpC)-/carbapenemases producing organisms [21]. It should be noted that confirmatory testing for ESBL production using phenotypic tests such as CDDT or molecular methods is recommended for 3GC-R organisms [22]. Additionally, several techniques used at the NHL for AST evaluation are not recommended in the CLSI M100 guideline. These include the use of oxacillin instead of cefoxitin for the detection of MRSA; and the disc diffusion method, instead of MIC methods (E test and broth dilution test), for the evaluation of vancomycin resistance [16]. In the former, experts have argued that cefoxitin is a better inducer of *mecA*-mediated resistance and that disc diffusion methods can overestimate vancomycin resistance [23].

Separately, we demonstrated a correlation between distance to the laboratory and pathogen recovery rates. In terms of distance with respect to NHL, Sembel and Haz Haz, which recorded the lowest recovery rates, are the farthest. This finding highlights a potential problem with existing specimen transport modalities. By and large, the

use of out-dated tests (a common practice in most CML in Sub-Saharan Africa), along with the inability to test for specific drug-pathogen combinations listed in the WHO Priority Level (critical) list (Carbapenem-resistant *Enterobacteriaceae)* needs argent re-evaluation.

Based on these findings, the need to build capacity is clearly highlighted. The decentralisation of CMS and the adoption of automated continuous monitoring blood culture instruments (BACTEC, VersaTREK, or VITEK) in the NHL can be recommended. Other options, atleast in the long term; may include the adoption of fast identification methods such as matrix-assisted laser resorption/ionization - time of flight mass spectroscopy (MALDI TOF MS); real-time polymerase chain reaction (RT-PCR) based methods; fluorescence in situ Hybridization (FISH) based methods; whole genome sequencing, and other molecular assays [24].

Another prominent finding, with a far-reaching implication for CML practice in Eritrea, was the incidence of blood culture contamination (BCC). In general, BCC in BSIs in LMICs is poorly researched [4]. In the limited number of published BSI literature with BCC information, considerable heterogeneity abounds with respect to the methods used to distinguish between true pathogens and contaminants [25–28]. For example, Berkeley et al.. (2005) employed an identity-based system which classified all isolates of *CoNs*, *Bacillus spp.*, *Micrococcus spp.*, and *viridans streptococci* isolates as contaminants regardless of the clinical picture. In contrast, Hattori et al. (2018) included episodes of BSIs with CoNs, *Bacillus* spp., *Corynebacterium* spp., and *Cutibacterium* spp [29].,. More recent algorithms rely on the number of positive cultures (isolation of commensals which are also known to be pathogenic in ≥ 2 culture sets) and clinical scenario [19]. In our analysis, positive cultures of GPB, *viridans streptococci*, and CoNs without AST data were characterized as potential contaminants. Based on this approach, potential contaminants represented 363 (35.4%) of BCI. Heterogeneity in the distribution of contaminants across hospitals was also observed– note that all isolates in hospitals other than OPH or ONRH were potential contaminants. Although the difference in methodology limits the comparability of the results across the region; our results suggest that BCC maybe a problem in the setting.

According to the existing literature, a high incidence of BCC can have several adverse clinical and financial consequences for patients and hospitals [30]. In particular, some authors have argued that it has the potential to undermine the utility of CML in patient care [31]. That is to say, some physicians / clinicians believe that BCC is the result of poor laboratory practice. Inexorably, this perception can create the sense that the results from a specific CML are unreliable. This assumption may, in turn, lead to under-utilization of CML services in these settings. Ironically, BCC occurs mainly during pre-analytical procedures such as sample collection and, to a lesser extent, during sample processing [31]. Therefore, the under-use of CML services in these settings points to the need for better collaboration between clinicians and laboratory personnel. To reduce the incidence of BCC to the required threshold (< 3%); expert opinion reiterates the need for prudent selection of patients; well-trained phlebotomy teams; surveillance and feedback; and the presence of multidisciplinary quality assurance teams, among others [31].

Although our analysis cannot provide comprehensive information on AMR resistance in Eritrea; it allows organism-level analysis that can allow us to assess the nature of the organism that circulates in specific hospitals in the country. As in other studies [28], CoNs was the predominant isolate in the respective scalar years (≤ 2016: 60 (48.4%) vs. ≥ 2020: 65(23.1%). The predominance of CoNs in this setting has multiple explanations, but only one imputation. In the first place, CoNs infections are particularly relevant as hospital-acquired or catheter-associated infectious agents and are a problem in preterm new-borns and /or neonates; elderly patients, critically ill patients, and, often, immunocompromised patients [32]. In our study, we believe that the low median age of the patients (median 1.2 years (IQR: 0.01 months – 15 years) implicates central line - associated blood stream infections given the fact that neonates are usually treated with central vein catheters.

Possible misclassification of non-pathogenic CoNs as pathogenic is another explanation for the high incidence of CoNs in most settings in the region. As mentioned previously, drawing a distinction between pathogenic vs. non-pathogenic CoNs in BCI is complicated as there is no single benchmark with adequate level of specificity [28]. In our study, the problem was compounded by several factors, which are relatively common in the region; including the use of a single culture set and the absence of patient and species-level data on CoNs. Note, for instance, that among the 41 or CoNs isolates; the only clinically relevant CoNs are *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus haemolyticus*, *Staphylococcus schleifieri*, *Staphylococcus xylosus*, *Staphylococcus hominis*, and *Staphylococcus lugdunensis* [28]. Therefore, species-level analysis is critical. All these, it must be emphasised; add uncertainty about the reliability of CoNs data from the region. To provide conclusive recommendations, additional research should be undertaken. Future research should leverage genomic technologies alongside better research designs.

Separately, analysis of the AST profiles of the 189 (34.3%) pathogenic CoNs demonstrated a high resistance to penicillin, 168 (92.3%); oxacillin, (171(94.5%); and gentamicin, 104 (56.5%). Moderate resistance to vancomycin

was also noted. Furthermore, we demonstrated that MDR resistance has increased overtime (≤2016: 25 (41.7%), vs. ≥2020: 50 (76.9%), *p*-value 0.001). High resistance to oxacillin has been reported in other studies [28]. Others have noted that horizontal transmission of genes such as the mecA gene (which mediates methicillin resistance and is located in SCCmec– a mobile genetic element) to other staphylococcal species is possible and that this can increase cross resistance against many antibiotics [33].

Among all BCIs analyzed, *Staphylococcus aureus*, one of the WHO's high-priority pathogens, was ranked 5th in frequency. Overtime, clear evidence of a significant decline in the isolation rate was also observed. Currently, the reasons for the observed decline remain unknown. In general, the low frequency of *Staphylococcus aureus* reported in this study is unusual for the region - it is the number 2 pathogen in most settings [34]. In line with previous studies in the country [35, 36]; we demonstrated a high resistance to TMP-SMZ, 26 (68.4%); penicillin, 38(86.4%); and oxacillin, 37(80.4%). Other notable AMR profiles included moderate resistance to ciprofloxacin, 19(45.2%); tetracyclines, 18(43.9%); erythromycin, 18 (42.9%) and gentamicin 14(31.1%). The high rate of resistance to commonly used antibiotics is a well-documented phenomenon in the region [37, 38] and has been linked, in the past; to a high frequency of MRSA. In general, MRSA usually has other resistance genes (e.g., determining resistance to gentamicin, sulphonamides, kanamycin, streptomycin, macrolides, fluoroquinolones or tetracyclines) and therefore can be classified as MDR [39].

Unlike previous studies [38], the frequency of VRSA was extremely high – 14(31.1%). In the past, the use of the disk diffusion method; which has a high incidence of false positivity, has been mentioned as a possible explanation [40]. Whether the foregoing explanation is true for these settings remains unknown. This non-resisting, MRSA and VRSA carry a serious prognosis, a recurrence rate, 5 – 10% [41]; high mortality rates; and the danger associated with their presence in any setting cannot be underestimated.

The main Gram (-ve) bacteria associated with BSIs were, in order of decreasing frequency: *Klebsiella spp.*, *Escherichia coli*, *Citrobacter spp*., *Enterobacter spp*., *Pseudomonas aeruginosa*, and *Salmonella spp*. Except for the period prior to <2014, Gram (-ve) predominated in subsequent scalar years. The prevalence of Enterobacterales in BSIs in LMICs is consistent with the findings of several studies [42–45]. In most of these studies, *Escherichia coli* or *Klebsiella spp.*, are the dominant Gram (-ve) organisms. In a recent systematic and meta-analytical review on BSIs in Ethiopia, Alemnew et al. reported a pooled prevalence of 7.04% and 1.69% for *Klebsiella spp.*, and *Escherichia coli*, respectively. In Egypt, *Escherichia coli* was the most frequently identified Gram (-ve) bacteria (35%), followed by *Klebsiella spp*. (13%) [46]. Others have reported a pooled prevalence of 129 (46.7%) for *Klebsiella spp.*, *versus* 128 (17.3%) for *Escherichia coli* in Vietnam [47]. Another notable result was the low prevalence of *Salmonella spp*., prior to <2019. This is contrary to reports from other regions (mostly South East Asia), where *Salmonella spp.*, is known to be a prominent pathogen in BSIs [48]. Altogether, there are few explanations and hypotheses for the remarkable variation in pathogen distribution in patients with BSIs in most settings.

In a separate analysis, we demonstrated that isolates of *Klebsiella spp.*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, and *Citrobacter spp.*, had a high resistance (ranging between 54.5% and 96.7%) to several first-line antibiotics, gentamicin; tetracycline, ampicillin, TMP-SMZ, cephalosporins (cephalexin, ceftazidime and ceftriaxone). In the last GLASS report (2022), the authors noted that a high rate of resistance to 3GC is generally associated with the presence of ESBL. Importantly, resistance to 3GC is known to be a critical determinant of poor outcomes - increasing the odds of death 15-fold in some settings [19]. Equally significant is the high resistance to TMP-SMZ. This is because TMP-SMZ resistance genes in enterobacterales are frequently associated with mobile genetic elements that increase the likelihood of pan drug resistance or extreme-drug-resistance [49].

When comparing our data with a systematic review of Gram (-ve) AMR in BSIs in resource-limited countries, we noted a number of striking similarities. For example, resistance of *Escherichia coli* to 3GC was comparable to what has been reported in other LMICs – 39(60.9%) vs. 58.3% (IQR 39.8–70.2) in LMICs [4]. In line with previous studies, amikacin, and to a lesser extent, ciprofloxacin; demonstrated better efficacy against Gram (-ve) bacteria. The relative effectiveness of amikacin in this setting may be due to its limited availability.

The problem of AMR was further compounded by the phenomenon of co-resistance, which enables isolates to be resistant to multiple agents by acquiring multiple mobile elements (transposons and plasmids) or chromosomal mutations. In this study, this phenomenon was evaluated using two indexes– the MDR and MAR index. Accordingly, MDR organisms were predominant, 472 (79.1%). Significant variations in MDR were observed between disparate etiological agents, with *Pseudomonas spp*., *Citrobacter spp*., and *Enterobacter spp.*, exhibiting rates >90%. Compared to other studies from the region (Kenya– Meru (46.3%) [50] Ethiopia– South Ethiopia (33.1%) [51]; Arba Minch (60.3%) [52] and Jimma (62.7%) [53]); the prevalence of MDR was higher (472 (79.1%): Of further concern, a significant increase in MDR rates was observed for some pathogens - *Acinetobacter spp*., and CoNs.

MDR or co-resistance, in general, has multiple imputations in any setting. Previous research has, for instance, shown that co-resistance can stymie AMR control strategies/policies which rely on restricting the use of antibiotics with high resistance rates. According to this position, co-resistance may lead to the persistence of resistance to specific antibiotics despite a drastic reduction in usage. For example, resistance to TMP-SMZ in *Escherichia coli* remains high in clinics across the United Kingdom despite a drastic reduction in its use [54]. The high prevalence of isolates which are co-resistant to ampicillin, gentamicin and 3GC has serious practical implications for current WHO guidelines for the treatment of neonatal sepsis (the guideline recommends the combined use of ampicillin or benzylpenicillin for 7–10 days with 2 doses of gentamicin as first-line empiric therapy [55]. Commenting on this problem, some authors have suggested that the high rate of co-resistance to ampicillin and gentamicin; and the observed species-level variance, points at the need for AST-guided antimicrobial therapy [44]. This is particularly important as the remaining therapeutic options such as meropenem, chloramphenicol (in some agents), and vancomycin (for Gram + ve bacteria) are relatively expensive and can trigger adverse reactions, particularly in infants; if not adequately monitored [55].

Finally, the MAR index has been described as a cost-effective and easy-to-use tool for monitoring selection pressure exerted by the use of antibiotics on bacterial isolates. For example, the MAR index > 2 is frequently observed in bacterial isolates from settings where exposure to antibiotics is high. Others have argued that a high MAR index highlights the potential misuse of broad-spectrum antibiotics in hospital environments. In this study, the MAR index was > 2 (mean (± SD) = 0.55 (± 0.23)) with wide species-level variation. Except for 2020, we also observed an increase in the overall MAR index over the duration of the study.

Similar to other studies [23], the decrease in the MAR index in 2020 can be explained by a number of factors, including random fluctuations in the number of samples and changes in healthcare practice. For instance, stringent measures including travel restrictions, changes in admission requirements (and attendant changes in patient mix), or the more stringent infection control measures associated with COVID-19 may account for the slight deepening of the MAR index in 2020. Using timed cluster density analysis, we demonstrated that resistotypes diversity has increased over time. Such increases, it has been argued, may not necessarily be due to the spread of a single gene cassette carrying multiple genes, but may be due to the spread of multiple phenotype co-resistance/multiple genetic determinants [56].

Strengths and limitations of the study

Our study has several biases and limitations. First, retrospective studies have some inherent limitations, including data quality issues and data missingness. Note, for instance, that the NHL logbook does not contain information on hospital acquired blood stream infections; primary or secondary bacteraemia; reference information; device-related blood stream infection; previous antibiotic use, sepsis, or other outcomes, among others. Second, the fact that our data was extracted from a laboratory register from a facility which sources samples from centralized tertiary level facilities imposes considerable limits on the representativeness and generalizability of our findings. Indeed, one might even argue that the age bias and year-to-year variations in the number of samples submitted by the respective institutions have more to do with vagaries of institutional practices (or other confounders) than with true epidemiological trends. Third, and in some instances, the use of out-dated techniques and lack of confirmatory testing means that our results should be interpreted with caution. Although these factors may limit the quality of our findings, they only affect a subset of the information provided. Furthermore, these concerns are relatively common in a large number of published studies from LMICs.

Conclusions

To our knowledge, this is the first study to report the epidemiology of BSIs in any setting in Eritrea, including the involvement of AMR microorganisms, MDR, and associated trends. The main findings included: limited coverage of AMR tests for BCIs; shortcomings in the performance of BCC at the NHL– a situation marked by unclear AST testing strategies; use of out-dated tests; limited range of media and high rate (potential) of BCC. Moreover, we noted a significant year-to-year variation in the burden of specific pathogens with an increasing tendency towards the isolation of the more problematic ESKAPE organisms. Additional findings included a high rate of resistance to first- and in second-line therapies and increasing burden of MDR. Further, MDR was characterized with respect to annualised changes; trends in the MAR index and specific pathogen– drug combinations (MRSA, 3GC-R, *Enterobacteriaceae*, VRSA, vancomycin-intermediate *Staphylococcus aureus*. The prevailing AMR scenario, it should be emphasized; is evolving against a background of extremely limited CML capacity; extreme reliance on empiric treatment; a lacuna in data management, and severely limited research efforts. Although there are many limitations and biases, this study clearly highlights a concerning level of AMR in Eritrea. At the most basic level, it emphasises the need for AST-guided therapy for BSIs. To address some of these concerns, improvements in the CML infrastructure and technical

capacity; AMR surveillance; infection control practices; and the development of a simple, easy-to-implement antibiotic stewardship program remain essential. Additionally, a better understanding of local antimicrobial susceptibility is an urgent issue. In particular, improved multicenter molecular epidemiology studies (preferably prospective) employing standardized protocols with data on underlying disease, treatment, and clinical outcomes are required.

Abbreviations

AST	Antimicrobial susceptibility testing
TMP-SMZ	Cotrimoxazole / trimethoprim - sulfur methoxazole
CLSI	Clinical and Laboratory Standards Institute
MIC	Minimal inhibition concentration
MAR index	Multiple antibiotic resistance
ECDPC	European Center for Disease Prevention and Control
GPB	Gram positive cultures
SPSS	Statistical Package for Social Sciences
IQR	interquartile range
ANOVA	Analysis of variance
MOH	Ministry of Health
CML	Clinical microbiology laboratory
CMS	Clinical microbiology services
CDDT	Combination disk diffusion test
BCC	Blood culture contamination
CVC	Central vein catheters
RT-PCR	Real-time polymerase chain reaction
FISH	Fluorescence in situ Hybridization
LOS	Length of stay
VISA	Vancomycin-intermediate Staphylococcus aureus
VRSA	Vancomycin Resistant Staphylococcus Aureus

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-025-00780-0.

Supplementary Material 1

Acknowledgements
The authors thank the laboratory staff of NHL who supported this work.

Author contributions
YGA, ETH, HHG, MHE, and AWA conceived and formulated the study design. YGA, ETH, HHG, MHE, and AWA performed data collection. OOA, YGA, AWA, and HHG supervised the data collection process and performed other administrative functions. OOA, STM, and MEH did the statistical analysis and data presentation. OOA, STM, AMB, EYG, BT, YGA, AWA, HHG, and MEH wrote and edited the first draft of the manuscript. All authors read and approved the final manuscript.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Financial support
Data were collected from the NHL laboratory report book and no financial support was obtained for the study.

Supporting documents
The data set supporting the conclusions of this article is available from the corresponding author on reasonable request.

Competing interests
The authors declare no competing interests.

Author details
[1]Microbiology Department, National Health Laboratory, Ministry of Health, Asmara, Eritrea
[2]National Blood Transfusion Center, Asmara, Eritrea
[3]Unit of Clinical Laboratory Science, Orotta College of Medicine and Health Science (OCMHS), Asmara, Eritrea
[4]Northern Red Sea Ministry of health branch, Nakfa Hospital, Asmara, Eritrea
[5]National Medicines and Food Administration, Ministry of Health, Asmara, Eritrea
[6]Department of Obstetrics and Gynecology, Orotta College of Medicine and Health Sciences, Orotta National Referral Maternity Hospital, Ministry of Health, Asmara, Eritrea
[7]Department of Medical Microbiology, Orotta College of Medicine and Health Sciences, Asmara, Eritrea

Received: 10 October 2024 / Accepted: 28 January 2025
Published online: 21 February 2025

References

1. Rudd KE, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the global burden of Disease Study. Lancet. 2020;395(10219):200–11.
2. Ikuta KS, et al. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the global burden of Disease Study 2019. Lancet. 2022;400(10369):2221–48.
3. Timsit J-F, et al. Blood stream infections in critically ill patients: an expert statement. Intensive Care Med. 2020;46(2):266–84.
4. Kim D, et al. Major blood stream infection-causing bacterial pathogens and their Antimicrobial Resistance in South Korea, 2017–2019: phase I report from Kor-GLASS. Front Microbiol. 2021;12:799084.
5. ECDC. *Healthcare-associated infections acquired in intensive care units.* Annual Epidemiological Report for 2017, 2019.
6. Shankar PR, Balasubramanium R. Antimicrobial resistance: global report on surveillance 2014. Australasian Med J (Online). 2014;7(5):237.
7. Allel K, et al. The impact of inpatient blood stream infections caused by antibiotic-resistant bacteria in low- and middle-income countries: a systematic review and meta-analysis. PLoS Med. 2023;20(6):e1004199.
8. Seboxa T, et al. High mortality from Blood Stream infection in Addis Ababa, Ethiopia, is due to Antimicrobial Resistance. PLoS ONE. 2015;10(12):e0144944.
9. Murray CJ, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55.
10. Manyahi J, et al. Multi-drug resistant bacteria predict mortality in blood stream infection in a tertiary setting in Tanzania. PLoS ONE. 2020;15(3):e0220424.
11. Tornimbene B, et al. Global Antimicrobial Resistance and Use Surveillance System on the African continent: early implementation 2017–2019. Afr J Lab Med. 2022;11(1):1–11.
12. Elton L, et al. Antimicrobial resistance preparedness in sub-saharan African countries. Antimicrob Resist Infect Control. 2020;9:1–11.
13. Diekema DJ, et al. The microbiology of blood stream infection: 20-year trends from the SENTRY antimicrobial surveillance program. Antimicrob Agents Chemother. 2019;63(7). https://doi.org/10.1128/aac. 00355– 19.
14. Tadesse BT, et al. Antimicrobial resistance in Africa: a systematic review. BMC Infect Dis. 2017;17(1):616.
15. Vernet G, et al. Surveillance for antimicrobial drug resistance in under-resourced countries. Emerg Infect Dis. 2014;20(3):434–41.
16. Weinstein MP, Lewis JS 2. nd, The Clinical and Laboratory Standards Institute Subcommittee on Antimicrobial susceptibility testing: background, Organization, functions, and processes. J Clin Microbiol, 2020. 58(3).
17. Magiorakos A-P, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.
18. Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983;46(1):165–70.

19. Semret M, et al. Prolonged empirical antibiotic therapy is correlated with blood stream infections and increased mortality in a tertiary care hospital in Ethiopia: bacteriology testing matters. JAC Antimicrob Resist. 2020;2(3):dlaa039.
20. Legba BB, et al. Assessment of blood cultures and antibiotic susceptibility testing for bacterial sepsis diagnosis and utilization of results by clinicians in Benin: a qualitative study. Front Public Health. 2022;10:1088590.
21. Muriuki CW, et al. Phenotypic and genotypic characteristics of Uropathogenic Escherichia coli isolates from Kenya. Microb Drug Resist. 2022;28(1):31–8.
22. Lester R, et al. Prevalence and outcome of blood stream infections due to third-generation cephalosporin-resistant Enterobacteriaceae in sub-saharan Africa: a systematic review. J Antimicrob Chemother. 2020;75(3):492–507.
23. Prevention ECfD, Control. Assessing the health burden of infections with antibiotic-resistant bacteria in the EU/EEA, 2016–2020. Stockholm: ECDC; 2022.
24. Costa SP, Carvalho CM. Burden of bacterial blood stream infections and recent advances for diagnosis. Pathogens Disease. 2022;80(1):ftac027.
25. Hill PC, et al. Bacteraemia in patients admitted to an urban hospital in West Africa. BMC Infect Dis. 2007;7:2.
26. Kirn T, Weinstein M. Update on blood cultures: how to obtain, process, report, and interpret. Clin Microbiol Infect. 2013;19(6):513–20.
27. Berkley JA, et al. Bacteremia among children admitted to a rural hospital in Kenya. N Engl J Med. 2005;352(1):39–47.
28. Asante J, et al. Review of clinically and epidemiologically relevant Coagulase-negative Staphylococci in Africa. Microb Drug Resist. 2020;26(8):951–70.
29. Hattori H, et al. Epidemiology and risk factors for mortality in blood stream infections: a single-center retrospective study in Japan. Am J Infect Control. 2018;46(12):e75–9.
30. Organization WH. Antimicrobial resistance: global report on surveillance. World Health Organization; 2014.
31. Doern GV, et al. Practical guidance for clinical microbiology laboratories: a comprehensive update on the problem of blood culture contamination and a discussion of methods for addressing the problem. Clin Microbiol Rev. 2019;33(1):00009–19. https://doi.org/10.1128/cmr.
32. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev. 2014;27(4):870–926.
33. Santella B, et al. Sepsis—A retrospective cohort study of blood stream infections. Antibiotics. 2020;9(12):851.
34. Kern W, Rieg S. Burden of bacterial blood stream infection—a brief update on epidemiology and significance of multidrug-resistant pathogens. Clin Microbiol Infect. 2020;26(2):151–7.
35. Garoy EY, et al. Magnitude of Multidrug Resistance among Bacterial isolates from Surgical Site infections in two National Referral hospitals in Asmara, Eritrea. Int J Microbiol. 2021;2021:p6690222.
36. Garoy EY, et al. Methicillin-Resistant Staphylococcus aureus (MRSA): prevalence and antimicrobial sensitivity pattern among Patients-A Multicenter Study in Asmara, Eritrea. Can J Infect Dis Med Microbiol. 2019;2019:p8321834.
37. Meremikwu MM, et al. Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. BMC Infect Dis. 2005;5:1–4.
38. Sorsa A, et al. Blood culture result profile and antimicrobial resistance pattern: a report from neonatal intensive care unit (NICU), Asella teaching and referral hospital, Asella, South East Ethiopia. Volume 8. Antimicrobial Resistance & Infection Control; 2019. pp. 1–6.
39. Ibrahim RA, et al. Identification of Escherichia coli from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Vet Res. 2019;15(1):159.
40. Belete MA, et al. The prevalence of Vancomycin-resistant Staphylococcus aureus in Ethiopia: a systematic review and meta-analysis. Volume 12. Antimicrobial Resistance & Infection Control; 2023. p. 86. 1.
41. Gradel KO, et al. Impact of appropriate empirical antibiotic treatment on recurrence and mortality in patients with bacteraemia: a population-based cohort study. BMC Infect Dis. 2017;17(1):122.
42. Alex V, Nana T, Chibabhai V. A retrospective analysis of community-onset blood stream infections at a tertiary-care academic hospital in South Africa. Are current empiric antimicrobial practices appropriate? Antimicrob Stewardship Healthc Epidemiol. 2021;1(1):e67.
43. Beshah D, et al. Antimicrobial resistance and associated risk factors of Gram-negative bacterial blood stream infections in Tikur Anbessa Specialized Hospital, Addis Ababa. Infection and Drug Resistance; 2022. pp. 5043–59.
44. Seni J, et al. Deciphering risk factors for blood stream infections, bacteria species and antimicrobial resistance profiles among children under five years of age in North-Western Tanzania: a multicentre study in a cascade of referral health care system. BMC Pediatr. 2019;19(1):32.
45. Musicha P, et al. Trends in antimicrobial resistance in blood stream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study. Lancet Infect Dis. 2017;17(10):1042–52.
46. Helmy AK, et al. Emergence of microbial infections in some hospitals of Cairo, Egypt: studying their corresponding antimicrobial resistance profiles. BMC Infect Dis. 2023;23(1):424.
47. Dat VQ, et al. Bacterial blood stream infections in a tertiary infectious diseases hospital in Northern Vietnam: aetiology, drug resistance, and treatment outcome. BMC Infect Dis. 2017;17:1–11.
48. Aliya Naheed AN et al. *Burden of typhoid and paratyphoid fever in a densely populated urban community, Dhaka, Bangladesh*. 2010.
49. Ramesh Kumar MR, et al. Dissemination of Trimethoprim-Sulfamethoxazole Drug Resistance genes Associated with Class 1 and class 2 integrons among Gram-negative Bacteria from HIV patients in South India. Microb Drug Resist. 2017;23(5):602–8.
50. Miriti DM, Muthini JM, Nyamache AK. Study of bacterial respiratory infections and antimicrobial susceptibility profile among antibiotics naive outpatients visiting Meru teaching and referral hospital, Meru County, Kenya in 2018. BMC Microbiol. 2023;23(1):172.
51. Gebre AB, Begashaw TA, Ormago MD. Bacterial profile and drug susceptibility among adult patients with community acquired lower respiratory tract infection at tertiary hospital, Southern Ethiopia. BMC Infect Dis. 2021;21(1):440.
52. Regasa B. Drug resistance patterns of bacterial pathogens from adult patients with pneumonia in Arba Minch hospital, South Ethiopia. J Med Microbiol Diagnosis. 2014;3(4):1.
53. Regasa B, et al. Antimicrobial susceptibility pattern of bacterial isolates from community-acquired pneumonia patients in Jimma University specialized hospital, Jimma, Ethiopia. Saudi J Health Sci. 2015;4(1):59–64.
54. Sundqvist M, et al. Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. J Antimicrob Chemother. 2010;65(2):350–60.
55. Fuchs A, et al. Reviewing the WHO guidelines for antibiotic use for sepsis in neonates and children. Paediatrics Int Child Health. 2018;38(sup1):S3–15.
56. Hayer SS et al. Genetic determinants of resistance to extended-spectrum cephalosporin and fluoroquinolone in Escherichia coli isolated from diseased pigs in the United States. mSphere, 2020. 5(5).

Publisher's note