

Commonly Isolated Bacteria from Sterile Body Fluid Samples and their Antibiotic Sensitivity Pattern in Belize – A Retrospective Pilot Study

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Abstract

*In developing countries like Belize, antimicrobial resistance presents unique public health challenges driven by limited surveillance resources, substandard or falsified antibiotics, inadequate testing facilities, indiscriminate human and agricultural antibiotic use, and weak policy enforcement. Multidrug-resistant bacterial pathogens pose a severe global threat, making the rapid identification of pathogens in sterile body fluids (SBF) essential for effective clinical management. To our knowledge, this study is the first of its kind in Belize to evaluate bacterial isolates and antimicrobial susceptibility patterns in SBF. Retrospective records from the Belize Central Medical Lab spanning August 2018 to July 2020 were analyzed. Positive cultures and antimicrobial susceptibility testing were performed using the Kirby-Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Coagulase-negative staphylococci were the most prevalent isolate (23%), likely representing skin contaminants, followed by *Pseudomonas aeruginosa* (15.5%). For gram-positive isolates, vancomycin demonstrated 100% susceptibility, whereas ampicillin and erythromycin showed low sensitivity rates of 16% and 32%, respectively. Gram-negative isolates exhibited high sensitivity to imipenem (94.5%) and amikacin (91%), but notable resistance to ciprofloxacin (24.3%). Additionally, pronounced resistance to gentamicin was observed across both gram-positive (31.7%) and gram-negative (35.4%) isolates. These findings provide essential baseline data to assist healthcare professionals in selecting appropriate empirical antibiotic therapies for bacterial infections in Belize. Ultimately, this work supports the design and implementation of larger-scale research aimed at strengthening national public health surveillance and policy enforcement strategies.*

Keywords: Antibiotic-resistant, antibiotic susceptibility, bacterial isolates, sterile body fluids, Belize

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Introduction

Multidrug-resistant bacterial pathogens in community and hospital settings are currently recognized as a significant public health threat globally (Munita & Arias, 2016). Resistant pathogenic bacteria have been detected and isolated from sterile body fluids, leading to severe morbidity and mortality in humans (Tsegay et al., 2019; Sandhya et al., 2019). Sterile body fluids (SBF) such as pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid, or aspirates from lymph nodes, brain, heart, liver, spleen, vitreous fluid, kidney, pancreas, ovary, and vascular tissue are free of microorganisms (fungi, parasites, viruses, and bacteria) (Mahon et al., 2015). However, a few studies have reported that pathogenic organisms such as *Neisseria meningitidis*, *Haemophilus influenzae*, *Klebsiella* species, *Staphylococcus aureus*, *Escherichia coli*, and others were found in SBF, with attendant life-threatening infections (Altun et al., 2015; Mahon et al., 2015; Munita & Arias, 2016). Infections caused by resistant pathogenic organisms lead to morbidity and mortality and impose a significant economic burden (WHO, 2014; Munita & Arias, 2016). Consequently, timely detection and rapid identification of pathogenic organisms in SBF are essential to primary management (Altun et al., 2015). In developing countries like Belize, antimicrobial resistance presents unique challenges such as a lack of resources for surveillance, the influx of substandard or fake antibiotics, lack of facilities for testing antibiotics quality, indiscriminate antibiotics usage in humans and for agriculture, lack of consistent policies on antibiotics usage or lack of enforcement of antibiotic policies (Tadesse et al., 2017; Da Silva et al., 2020; Husaini et al., 2020; Husaini et al., 2023). Bacteria such as *Pseudomonas* spp., *Escherichia coli*, *Acinetobacter* spp., and *Klebsiella* spp. etc. may cause infections in various SBFs, and several of these bacteria have been reported to be resistant to some antibiotics (WHO, 2000; Mahon et al., 2015; Nwachukwu et al., 2026). Since antibiotics drive multidrug resistance, there is an urgent need to implement antibiotic susceptibility surveillance to prevent the emergence of resistant bacteria and to support efficient infection control, especially in developing countries (Tsegay et al., 2019). This study was conducted in Belize, Central America. The population of Belize is currently slightly above 400,000 persons (SIB, n.d.). To date, no study has been conducted or published on bacterial isolates and antimicrobial susceptibility patterns from sterile body fluids in the country. Hence, evaluating the bacterial profile and antibiotic susceptibilities in SBF is significant for providing baseline data for Belize to inform the design, development, and implementation of large-scale research, leading to strategies for the surveillance and strengthening of public health.

Materials and Methods

Study design and Area

Records of consecutive diagnoses of bacterial infection of SBF from August 2018 to July 2020 at Belize Central Medical Lab were studied retrospectively to determine the bacterial isolates and pattern of bacterial susceptibility in SBF. The study design of Tsegay et al. (2019) was adapted for this study. The University of Belize Faculty of Health Sciences approved the study protocol.

Sample and sampling technique

Sociodemographic and clinical data were obtained from 187 culture-positive samples using a prepared data abstraction format, focusing on patient age, gender, district of residence, type of body fluid, isolated bacteria, antibiotic sensitivity test results, and antibiotic discs tested. The records indicate that culture and sensitivity tests were performed on all sterile body fluids. Controls, which validate the testing process, were used at every stage of testing to ensure accurate isolation and identification of the microorganism and to determine its response to antimicrobial agents. Positive cultures and their antimicrobial susceptibility testing are performed in accordance with the guidelines of the Clinical and Laboratory Standards Institute

(CLSI, 2020). All bacterial isolates are examined for colony characteristics, Gram reaction, motility, and biochemical tests. The collected specimens were cultured on Blood agar and MacConkey agar for Gram-negative and Gram-positive organisms, and on chocolate agar for fastidious organisms. The agar used was BD Difco agar, whereas the susceptibility discs were BD BBL Sensi-Discs. Antimicrobial susceptibility testing is performed using the Kirby-Bauer Disc Diffusion method (CLSI, 2020). Furthermore, the susceptibilities of gram-negative bacteria isolated from sterile body fluids to amikacin, ciprofloxacin, gentamicin, imipenem, and piperacillin/tazobactam were evaluated. In contrast, for Gram-positive, ampicillin, ciprofloxacin, erythromycin, gentamicin, tetracycline, and vancomycin were assessed using reference strains as quality control for culture and susceptibility testing.

Data analysis

The Statistical Package for the Social Sciences (SPSS) version 22 was used to analyze the data after checking for accuracy and completeness. Chi-square was used to assess associations between variables in SBF, with p-values < 0.05 considered significant in all analyses.

Results

In SBF, one hundred eighty-seven (187) samples yielded bacterial isolates and were consequently evaluated for antimicrobial susceptibility. The Gram reactions of isolated bacterial pathogens in SBF revealed that most were Gram-negative (n=98; 52.4%). The gram-negative organisms isolated include *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Acinetobacter* spp., *Proteus mirabilis*, *Serratia marcescens*, and *Morganella morganii*. The gram-positive isolates were gram-positive cocci and accounted for 40.1% (n=75). They include Coagulase-negative staphylococci, *Staphylococcus aureus*, *Enterococcus faecalis*, and Group B streptococcus.

The demographic results for 187 specimens that yielded isolates showed that the majority of participants were under 1 year of age (n=46, 24.6%), with males (n=106, 56.7%) being the most common. No significant differences were observed across age groups or between genders. The Belize district has the highest number of samples that yielded isolates. Population-wise, the Belize district has the highest in the country (Table 1).

Table 1. Demographic distribution of samples

Age (years)	Frequency	%
Below 1 year	46	24.6
1-14	14	7.4
15-24	22	11.8
25-34	23	12
35-44	24	12.8
45-54	14	7.5
55-64	24	12.9
65 & above	20	10.7
Gender		
Female	81	43.3
Male	106	56.7
District		
Belize	81	43.3
Cayo	41	21.9

Corozal	13	7.0
Orange Walk	12	6.4
Stann Creek	30	16.0
Toledo	10	5.3

Table 2 shows the isolation rate of each bacterial pathogen in cerebrospinal fluid (CSF), peritoneal fluid, pleural fluid, and other body fluids. It also shows the prevalence of specimen types, with “other fluids” making up the majority (n=153, 81.8%), followed by cerebrospinal fluid (n=22, 11.8%), peritoneal fluid (n=6, 3.2%), and pleural fluid (n=6, 3.2%). The most common bacterial isolate was Coagulase-negative Staphylococci (23.0%), followed by *Pseudomonas aeruginosa* (15.5%), *Klebsiella pneumoniae* (13.9%), *Escherichia coli* (9.0%), *Staphylococcus aureus* (7.0%), and *Enterococcus faecalis* (7.0%).

Table 2. Demographic distribution of samples

		Sterile Fluid					Total (n =187) n (%)
		Gram Reaction	CSF n (%)	Peritoneal n (%)	Pleural n (%)	Other n (%)	
Pathogen	<i>Acinetobacter spp.</i>	Gram-Negative	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.0)	3 (1.6)
	<i>Candida albicans</i>	Fungi	1 (4.5)	2 (33.0)	0 (0.0)	2 (1.3)	5 (2.7)
	CoNs*	Gram-Positive	2 (9.1)	1 (16.7)	1 (16.7)	39 (25.5)	43 (23.0)
	<i>Cryptococcus neoformans</i>	Fungi	6 (27.4)	0 (0.0)	0 (0.0)	0 (0.0)	6 (3.3)
	<i>Enterobacter cloacae</i>	Gram-Negative	3 (13.6)	0 (0.0)	0 (0.0)	7 (4.6)	10 (5.3)
	<i>Enterococcus faecalis</i>	Gram-Positive	2 (9.1)	0 (0.0)	0 (0.0)	11 (7.2)	13 (7.0)
	<i>Escherichia coli</i>	Gram-Negative	2 (9.1)	1 (16.7)	0 (0.0)	14 (9.2)	17 (9.0)
	<i>Klebsiella pneumoniae</i>	Gram-Negative	1 (4.5)	0 (0.0)	0 (0.0)	25 (16.3)	26 (13.9)
	<i>Morganella morganii</i>	Gram-Negative	1 (4.5)	0 (0.0)	0 (0.0)	3 (2.0)	4 (2.1)
	<i>Neisseria gonorrhea</i>	Gram-Negative	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.5)
	<i>Proteus mirabilis</i>	Gram-Negative	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.6)	4 (2.1)
	<i>Pseudomonas aeruginosa</i>	Gram-Negative	2 (9.1)	2 (33.0)	1 (16.7)	24 (15.7)	29 (15.5)
	<i>Serratia marcescens</i>	Gram-Negative	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.6)	4 (2.1)
	<i>Staphylococcus aureus</i>	Gram-Positive	0 (0.0)	0 (0.0)	3 (50.0)	10 (6.5)	13 (7.0)
	Streptococcus Group B	Gram-Positive	2 (9.1)	0 (0.0)	0 (0.0)	4 (2.6)	6 (3.3)

Other Bacterial Pathogens	Undetermined	0 (0.0)	0 (0.0)	1 (16.7)	2 (1.3)	3 (1.6)
Total Specimen <i>n</i> (%)		22 (11.8%)	6 (3.2%)	6 (3.2%)	153 (81.8%)	187 (100%)

*Coagulase-negative Staphylococci

Table 3 shows the antibiotic susceptibility patterns of the gram-positive bacteria isolated from sterile body fluids, where AMP=ampicillin, CIP=ciprofloxacin, ERY=erythromycin, GEN=gentamycin, TET=tetracycline, VAN=Vancomycin, R=resistant, I=intermediate, and S=susceptible. Not all isolates were tested against all listed antibiotics. Based on the total susceptibility percentage alone from the isolated tested, effective drugs for Gram-positive isolates are Vancomycin (100%) and ampicillin (80%). The remaining antibiotics were Ciprofloxacin (70.4%), Erythromycin (68.0%), Tetracycline (68%), and Gentamycin (58.5%). The resistance rates for the Gram-positive isolates are 16% and 32% to ampicillin and erythromycin, respectively.

Table 3: Antimicrobial susceptibility pattern of gram-positive bacteria isolated from sterile body fluids.

Bacterial Isolate	Pattern	AMP N (%)	CIP N (%)	ERY N (%)	GEN N (%)	TET N (%)	VAN N (%)
<i>Coagulase Negative Staphylococci</i>	R	2 (40.0)	4 (28.6)	2 (33.3)	3 (20.0)	1 (16.7)	0 (0.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	S	3 (60.0)	10 (71.4)	4 (66.7)	12 (80.0)	5 (83.3)	5 (100.0)
<i>Enterococcus faecalis</i>	R	1 (7.7)	0 (0.0)	0 (0.0)	8 (61.5)	0 (0.0)	0 (0.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	4 (30.8)	0 (0.0)	0 (0.0)
	S	12 (92.3)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	13 (100.0)
<i>Staphylococcus aureus</i>	R	1 (100.0)	3 (23.1)	6 (46.2)	2 (15.4)	0 (0.0)	0 (0.0)
	I	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	2 (15.4)	0 (0.0)
	S	0 (0.0)	9 (69.2)	7 (53.8)	11 (84.6)	11 (84.6)	13 (100.0)
<i>Group B Streptococcus</i>	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (83.3)	0 (0.0)
	I	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	S	5 (83.3)	0 (0.0)	6 (100.0)	0 (0.0)	1 (16.7)	0 (0.0)
Total	R	4 (16.0)	7 (25.9)	8 (32.0)	13 (31.7)	6 (24.0)	0 (0.0)
	I	1 (4.0)	1 (3.7)	0 (0.0)	4 (9.8)	2 (8.0)	0 (0.0)
	S	20 (80.0)	19 (70.4)	17 (68.0)	24 (58.5)	17 (68.0)	31 (100.0)

Table 4 illustrates the antibiotic susceptibility patterns of the gram-negative bacteria isolated in sterile body fluids, where AMK=amikacin, CIP=ciprofloxacin, GEN=gentamycin, IPM=imipenem, TZP=Piperacillin/Tazobactam, R=resistant, I=intermediate, and S=susceptible. Notably, not all isolates were tested against all listed antibiotics, which affected the total susceptibility percentages. Among the gram-negative isolates tested, the highest sensitivity rates were observed with Imipenem (94.5%) and Amikacin (91%), followed by Piperacillin/Tazobactam (81.4%), Ciprofloxacin (70.3%), and Gentamicin

(60.8%). The resistance patterns of gram-negative isolates varied, showing 4.1% to imipenem, 4.5% to amikacin, 8.6% to Piperacillin/Tazobactam, 24.3% to ciprofloxacin, and 35.4% to gentamycin.

Table 4: Antimicrobial susceptibility pattern of gram-negative bacteria isolated from sterile body fluids.

Bacterial Isolate	Pattern	AMK N (%)	CIP N (%)	GEN N (%)	IPM N (%)	TZP N (%)
<i>Acinetobacter spp</i>	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	I	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	S	3 (75.0)	2 (100.0)	4 (100.0)	2 (100.0)	2 (100.0)
<i>Enterobacter spp</i>	R	0 (0.0)	3 (60.0)	4 (66.7)	0 (0.0)	0 (0.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)
	S	6 (100.0)	2 (40.0)	2 (33.3)	6 (100.0)	5 (83.3)
<i>Escherichia coli</i>	R	0 (0.0)	2 (14.3)	3 (21.4)	0 (0.0)	0 (0.0)
	I	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)
	S	14 (93.3)	12 (85.7)	11 (78.6)	12 (100.0)	10 (90.9)
<i>Klebsiella pneumonia</i>	R	0 (0.0)	11 (57.9)	14 (66.7)	0 (0.0)	0 (0.0)
	I	0 (0.0)	3 (15.8)	0 (0.0)	1 (4.8)	4 (21.1)
	S	20 (100.0)	5 (26.3)	7 (33.3)	20 (95.2)	15 (78.9)
<i>Morganella morganii</i>	R	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
	I	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
	S	3 (100.0)	2 (66.7)	2 (66.7)	2 (100.0)	3 (100.0)
Other Gram-Negative Organisms	R	1 (33.3)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	S	2 (66.7)	3 (100.0)	2 (66.7)	2 (66.7)	2 (66.7)
<i>Proteus mirabilis</i>	R	0 (0.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)
	I	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	S	3 (75.0)	3 (75.0)	3 (75.0)	4 (100.0)	4 (100.0)
<i>Pseudomonas aeruginosa</i>	R	1 (3.4)	1 (5.0)	2 (10.0)	2 (10.0)	3 (16.7)
	I	0 (0.0)	0 (0.0)	3 (15.0)	0 (0.0)	0 (0.0)
	S	28 (96.6)	19 (95.0)	15 (75.0)	18 (90.0)	15 (83.3)
<i>Serratia marcescens</i>	R	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)	2 (50.0)
	I	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)
	S	1 (25.0)	4 (100.0)	2 (50.0)	4 (100.0)	1 (25.0)
Total	R	4 (4.5)	18 (24.3)	28 (35.4)	3 (4.1)	6 (8.6)
	I	4 (4.5)	4 (5.4)	3 (3.8)	1 (1.4)	7 (10.0)
	S	80 (91.0)	52 (70.3)	48 (60.8)	70 (94.5)	57 (81.4)

Table 5: Distribution of Bacterial Isolate in each sterile fluid by Gram Reaction/Identity

		Sterile Fluid				Total (n =187) n (%)	
		CSF n (%)	Peritoneal n (%)	Pleural n (%)	Other n (%)		
Pathogen	<i>Candida albicans</i>	1 (4.5)	2 (33.0)	0 (0.0)	2 (1.3)	5 (2.7)	Microbes of fungal origin n=11 (5.9%)
	<i>Cryptococcus neoformans</i>	6 (27.4)	0 (0.0)	0 (0.0)	0 (0.0)	6 (3.3)	
	<i>Enterobacter cloacae</i>	3 (13.6)	0 (0.0)	0 (0.0)	7 (4.6)	10 (5.3)	

	<i>Acinetobacter spp</i>	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.0)	3 (1.6)	All are <i>Enterobacteriaceae</i> except <i>P. aeruginosa</i>
	<i>Escherichia coli</i>	2 (9.1)	1 (16.7)	0 (0.0)	14 (9.2)	17 (9.0)	
	<i>Klebsiella pneumoniae</i>	1 (4.5)	0 (0.0)	0 (0.0)	25 (16.3)	26 (13.9)	
	<i>Morganella morganii</i>	1 (4.5)	0 (0.0)	0 (0.0)	3 (2.0)	4 (2.1)	
	<i>Serratia marcescens</i>	0 (0.0)	0 (0.0)		4 (2.6)	4 (2.1)	
	<i>Proteus mirabilis</i>	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.6)	4 (2.1)	
	<i>Pseudomonas aeruginosa</i>	2 (9.1)	2 (33.0)	1 (16.7)	24 (15.7)	29 (15.5)	GNBs (Gram-negative bacilli) n=97 (51.9%)
	<i>Neisseria gonorrhea</i>	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.5)	Gram-negative diplococci n=1 (0.5%)
	CoNs*	2 (9.1)	1 (16.7)	1 (16.7)	39 (25.5)	43 (23.0)	GPCs (Gram-positive cocci) n=75 (40.1%)
	<i>Staphylococcus aureus</i>	0 (0.0)	0 (0.0)	3 (50.0)	10 (6.5)	13 (7.0)	
	<i>Enterococcus faecalis</i>	2 (9.1)	0 (0.0)	0 (0.0)	11 (7.2)	13 (7.0)	
	Streptococcus Group B	2 (9.1)	0 (0.0)	0 (0.0)	4 (2.6)	6 (3.3)	
	Other Bacterial Pathogens	0 (0.0)	0 (0.0)	1 (16.7)	2 (1.3)	3 (1.6)	Bacteria of uncertain identity. n=3 (1.6%)
Total Specimen n (%)		22 (11.8%)	6 (3.2%)	6 (3.2%)	153 (81.8%)	187 (100%)	

Discussion

This study examined one hundred and eighty-seven (187) culture-positive body fluid specimens, with the majority (24.6) of the samples from children below the age of 1 (Table 1). "Other sterile body fluid" constituted 81.8%, CSF 11.8%, and peritoneal and pleural fluid samples 3.2% each (Table 2 & 5). "Other sterile body fluids" not categorized might include synovial fluid, ascitic fluid, amniotic fluid, or aspirates from lymph nodes, the brain, heart, liver, spleen, vitreous fluid, kidney, pancreas, ovary, or vascular tissue. Contrary to the findings of the current study, Tsegay et al. (2019) reported that most of the isolated bacteria came from CSF (47.7%), followed by pleural fluid (27.3%) and other sterile fluids (synovial and ascetic) at 25%. If the "other body fluids" in this study were separated into their respective groups, CSF might then become the most common specimen type. In this regard, further studies using specific specimen samples are recommended to determine the uniqueness of each body fluid.

The most common bacterial isolates in sterile body fluids are presented in Tables 2 and 5. Among the 'other body fluids,' Coagulase-negative staphylococci (CoNS) were the most common (25.5%). In comparison, *Cryptococcus neoformans* was the most commonly isolated organism from cerebrospinal fluid (CSF; 27.4%), with *Enterobacter cloacae* the most commonly isolated Gram-negative bacterial pathogen from CSF (13.6%). On the other hand, *Pseudomonas aeruginosa* was the most common organism in peritoneal fluid (33.0%), while *Staphylococcus aureus* was the most common bacterial agent of disease in pleural fluid, with an isolation rate of 50%.

From this study, gram-positive bacteria had an isolation rate of 40.1% (n=75) compared to gram-negative organisms of 52.4% (n=98), 5.9% (n=11) for fungi (*C. albicans*, n=5; *C. neoformans*, n=6), and 1.6% (n=3) for bacteria of uncertain identity. The result of this study does not fully align with the findings of Tsegay et al. (2019), which reported gram-positive isolates at 52.2% and gram-negative isolates at 47.7%. Although the study by Rouf & Nazir (2019) reported a preponderance of gram-negative organisms (70%) over gram-positive organisms (30%), which tends to support the present study's results, the values obtained are markedly different. An explanation for this dissimilarity could be the difference in the geographical location of their studies and differences in analytical and sampling emphasis.

Furthermore, the results of this study showed that the most commonly isolated bacteria were Coagulase-negative staphylococci (23.0%), followed by *Pseudomonas aeruginosa* (15.5%), *Klebsiella pneumoniae* (13.9%), *Escherichia coli* (9.0%), *Staphylococcus aureus* (7.0%), and *Enterococcus faecalis* (7.0%). Vishalakshi et al. (2016) found that *Staphylococcus aureus* was the most common (70%) isolated bacterium, followed by Coagulase-negative staphylococci, which includes *S. epidermidis*, a known skin flora. Coagulase-negative staphylococci are typically harmless outside the body; however, when present in the bloodstream or other sterile fluids, they can cause severe infections (Becker et al., 2014; Wasihun et al., 2015). Although some of the coagulase-negative staphylococci observed in this study may be due to skin contamination from improper specimen collection and handling, the controls and checks in place for the handling of sterile body fluids suggest that these may be true pathogens.

With regards to gram-negative bacteria, the organisms isolated in this study included *Pseudomonas aeruginosa* (15.5%) – a non-fermentative gram-negative bacterium, followed by *Klebsiella pneumoniae* (13.9%), *Escherichia coli* (9.0%), *Enterobacter cloacae* (5.3%), *Proteus mirabilis* (2.1%), *Serratia marcescens* (2.1%), *Morganella morganii* (2.1%), *Acinetobacter* spp (1.6%), and *Neisseria gonorrhoeae* (1.6%). Gram-positive isolates included Coagulase-negative staphylococci (23.0%), *Enterococcus faecalis* (7.0%), *Staphylococcus aureus* (7.0%), and Group B Streptococcus (3.3%). Due to regional differences, it is unsurprising that our data differ from those of a study in Northern India, in which Rouf & Nazir (2019) reported that *Staphylococcus aureus* was the most frequently isolated Gram-positive bacterium, with an isolation rate of 12.5%, while *Escherichia coli* was the most frequently isolated Gram-negative bacterium.

Table 3 shows Gram-positive susceptibility patterns. The Gram-positive isolates were tested for susceptibility to Ampicillin (AMP), Ciprofloxacin (CIP), Erythromycin (ERY), Gentamicin (GEN), Tetracycline (TET), and Vancomycin (VAN). Each of these antibiotics belongs to a different class; some, however, share similar mechanisms of action. For instance, Erythromycin, Tetracycline, and Gentamycin each act on bacteria by inhibiting protein synthesis (Mahon et al., 2015). Generally, Vancomycin is a "last-resort" drug used only after prior treatment has failed for infections caused by methicillin-resistant *Staphylococcus aureus* and *Enterococci* (Hu et al., 2013). Only a few Coagulase-negative Staphylococci (CoNS) isolates were tested for antibiotic susceptibility, likely because most of these isolates were suspected to be skin contaminants based on culture characteristics. A few of the CoNS tested showed the following sensitivity to Vancomycin (5/5; 100%), Tetracycline (5/6; 83.3%), and Gentamicin (12/15; 80.0%). All thirteen (13) of the *E. faecalis* isolates were tested for susceptibility against Vancomycin, ampicillin, and gentamicin, to which they showed 100%, 92.3%, and 7.7% susceptibility rates, respectively. Enterococci are intrinsically resistant to low aminoglycoside concentrations due to inefficient active transport across the cytoplasmic membrane, which may explain their low susceptibility to gentamicin (Sparo et al., 2018).

Moreover, all thirteen (13) *Staphylococcus aureus* isolates were tested against all six (6) antibiotics, showing the highest susceptibilities to Vancomycin (100%), Gentamicin (84.6%), and Tetracycline (84.6%). Globally, there has been a report of decreasing susceptibility of *S. aureus* to Vancomycin (Hu et al., 2013). In this study, however, none of the *S. aureus* organisms isolated from sterile body fluids showed resistance to Vancomycin. The possible explanations for this occurrence include effective infection control or an antibiotic treatment policy that reserves Vancomycin as a treatment option only for severe, multidrug-resistant staphylococcal or enterococcal infections. This finding supports the view that Vancomycin can

remain a last resort in Belize for treating *Staphylococcus aureus* infections in body fluids when other antibiotic classes, such as gentamicin, prove ineffective. Group B streptococci (GBS) isolates tested against erythromycin, ampicillin, and tetracycline showed 100%, 83.3%, and 16.7% susceptibility, respectively. The high rate of tetracycline resistance in Group B streptococcus (83.3%) is consistent with previous findings that GBS isolates are resistant to tetracycline (Sodani & Hawaldar, 2020).

The most effective drug for the gram-positive isolates appears to be Vancomycin (100%), followed by Ampicillin (80%), Ciprofloxacin (70.4%), Erythromycin (68%), Tetracycline (68%), and Gentamycin (58.5%) [Table 3]. Previous studies reported similar findings that gram-positive isolates from sterile body fluids were susceptible to Vancomycin (Rouf & Nazir, 2019; Sharma et al., 2017; Vishalakshi et al., 2016; Shume et al., 2022). Conversely, gram-positive isolates were reported to be sensitive to ciprofloxacin (70%), with resistance rates ranging from 16% for ampicillin to 32% for Erythromycin (Sharma et al., 2017). Interestingly, this is not only consistent but also an identical finding to the current study.

The gram-negative susceptibility patterns are presented in Table 4. The gram-negative isolates from sterile body fluids in Belize were tested for susceptibility to Amikacin (AMK), Ciprofloxacin (CIP), Gentamicin (GEN), Imipenem (IMP), and Piperacillin/Tazobactam (TZP). Not all isolates were tested against all listed antibiotics. Although gentamicin is the most used aminoglycoside, amikacin is particularly effective against resistant organisms (Thy et al., 2023). The present study showed that gram-negative isolates were much more susceptible to amikacin (91%) than gentamicin (60.8%). Until recently, Carbapenems such as imipenem and meropenem have been used as last-resort antibiotics for managing multidrug-resistant bacterial infections, though several bacterial species are becoming increasingly resistant due to the expression of enzymes that degrade carbapenems, known as carbapenemases (Meletis, 2016).

Pseudomonas aeruginosa was the predominant gram-negative isolate, and from the isolates tested, they showed high sensitivity rates to Amikacin (28/29; 96.6%), Ciprofloxacin (19/20; 95%), and Imipenem (18/20; 90%), followed by Piperacillin/Tazobactam (15/18; 83.3%), and Gentamicin (15/20; 75%) [Table 4]. The second most isolated gram-negative bacteria, *Klebsiella pneumoniae*, showed high sensitivity rates to Amikacin (20/20; 100%), Imipenem (20/21; 95.2%), and Piperacillin/Tazobactam (15/19; 78.9%). Furthermore, *Escherichia coli* showed high sensitivity to Imipenem (12/12; 100%), Amikacin (14/15; 93.3%), and Piperacillin/Tazobactam (10/11; 90.9%).

In terms of the overall performance of the antibiotics used against gram-negative isolates, the highest sensitivity was observed with Imipenem (70/74; 94.5%) and Amikacin (80/88; 91%), followed by Piperacillin/Tazobactam (57/70; 81.4%), Ciprofloxacin (52/74; 70.3%), and Gentamycin (48/79; 60.8%) similar to that described in other studies (Rouf & Nazir, 2019; Sharma et al., 2017). Gram-negative organisms showed higher sensitivity to aminoglycosides, which correlates well, as amikacin showed the second-highest sensitivity rate (91%) (Vishalakshi et al., 2016). Gentamicin, however, showed the highest rate of resistance (35.4%) among gram-negative isolates in sterile body fluids in Belize, according to this study. The resistance patterns of gram-negative isolates ranged from 4.1% to imipenem, 4.5% to amikacin, 8.6% to Piperacillin/Tazobactam, 24.3% to ciprofloxacin, and 35.4% to gentamicin, in increasing order of resistance. Because imipenem is one of the last-resort antibiotics, its higher sensitivity rate across all gram-negative isolates (94.5%) is unsurprising. This finding suggests that imipenem can continue to be used as an effective final resort antibiotic. Aminoglycosides such as amikacin may be useful as an empiric treatment option for gram-negative infections of sterile body fluids.

Conclusions

The retrospective prevalence of bacterial isolates from normally sterile body fluids in Belize was examined to identify their antibiotic susceptibility patterns from August 2018-July to 2020. In this study, 52.4% (n=98) of the isolates were gram-negative bacteria, 40.1% (n=75) were gram-positive bacteria, 5.9% (n=11) were fungi, and 1.6% (n=3) were bacteria of uncertain identity. The predominant isolated bacteria were Coagulase-negative staphylococci, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis*. However, Coagulase-negative staphylococci were the most prevalent isolates, probably due to skin contaminants; the most prevalent pathogenic isolate was *Pseudomonas aeruginosa*. Gram-positive isolates were found to be most sensitive to Vancomycin, whereas

the gram-negative isolates showed the highest sensitivity to Imipenem and Amikacin. Both gram-positive and gram-negative isolates showed the lowest susceptibility rates to gentamicin. The results of this retrospective pilot study can serve as a foundation for larger prospective studies that can guide laboratory scientists and policymakers in closely monitoring bacterial isolates and antimicrobial susceptibility patterns in sterile body fluids in Belize.

Limitations of the study

This study was carried out during the SARS-CoV-2 pandemic, with a few limitations. First, the retrospective data employed provided a faster way to obtain baseline data on the performance of commonly used antibacterial drugs against pathogens commonly isolated from sterile body fluids and, by extension, common bacterial pathogens isolated from hospitalized patients in Belize. Retrospective data have the disadvantages of potential confounders, limited control over the exposure factor, and limited control over covariates. The implications of this are that sampling and analytical errors may have affected the accuracy and reliability of the findings. Second, false-negative results may not be ruled out for nutritionally demanding and fragile pathogens because agar media, rather than broth, were used to isolate them. It was also most likely that disinfection failures at sample collection sites were responsible for the high isolation rate of the common skin commensals, the Coagulase-negative staphylococci (CoNS). In addition, because no anaerobic cultures were performed on the samples used in this study, most anaerobic pathogenic organisms were not isolated and therefore not accounted for. Finally, only a limited number of antibacterial drugs were used because no antibiotic policy was clearly defined. Despite these limitations, to our knowledge, this study is the first to report on bacterial isolates and antimicrobial susceptibility patterns in sterile body fluids in Belize, which is a strength of the study.

Declarations

Ethics approval and consent to participate.

The study protocol was approved by the Faculty of Health Sciences at the University of Belize.

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

IEN: Conceptualized the study, provided supervision, reviewed, and edited the first draft.

DCH: Conceptualized the study, provided supervision, reviewed, edited, and worked on the final draft.

ZEEA: Conducted literature search, data curation, analysis, and wrote the first draft.

RSC: Conducted literature search, data curation, analysis, and wrote the first draft.

ECP: Conducted literature search, data curation, analysis, and wrote the first draft.

All the authors read and approved the final manuscript.

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