Research Article

Antimicrobial Resistance Patterns of Foodborne Bacterial Isolates from HIV/AIDS Patients in Lusaka, Zambia

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Antimicrobial resistance is a major global public health concern and a food safety issue resulting in potential treatment failure, loss of treatment options, and increased likelihood and severity of disease. In this cross-sectional study, a total of 54 stool samples were obtained from respondents who are HIV-positive attending UTH. Microbiological identification of the bacteria from stool samples was done through culturing, and antimicrobial resistance patterns were studied through antimicrobial susceptibility testing. A total of 77 bacteria were isolated, and 36 of these were foodborne pathogens. Most of the isolates (92%) were resistant to ampicillin, followed by 80% resistant to sulfamethoxazole/trimethoprim. Foodborne bacteria such as *Staphylococcus aureus* isolates were 100% resistant to azithromycin and 90.9% resistant to methicillin, while the *Salmonella paratyphi* isolate was 100% resistant to ampicillin, sulfamethoxazole/trimethoprim, and amoxicillin/clavulanic acid. MDR (multidrug resistance) was seen in 19.4% of the isolates and XDR (extended drug resistance) in 27.8% of the foodborne bacterial isolates. Moreover, MDR foodborne bacteria were significantly associated with inpatients (*p-value*= 0.007). This study has revealed that MDR and XDR foodborne bacteria are at an alarming incidence in HIV/AIDS patients. Therefore, proper management of antimicrobial resistance and securing food safety should be timely issues to be focused on and resolved.

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Introduction

Food is defined as any substance that is consumed for nutritional support by an organism. Although it provides the body with essential nutrition, when contaminated by pathogens, it can result in foodborne diseases (FBD)[1]. Among the FBD, bacterial foodborne diseases (BFBD) are of specific importance to food safety and occur from ingesting bacterial contaminants that are responsible for causing mild to severe health conditions[2]. The main symptoms of BFBD include diarrhea, stomach pain, nausea, vomiting, and fever. They can last for a few hours or several days, with serious health problems and long-term effects including prolonged hospitalization and mortality[3]. Each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420,000 deaths, with the highest prevalence of 91 million cases of sickness recorded yearly and 137,000 deaths coming from Africa alone[4]. The severity of the illness highly depends on the immunity of individuals as well as the type of bacteria that contaminated the food. Immunocompromised individuals associated with a greater risk of foodborne infection (FBI) include people with human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS), pregnant women, people who have undergone organ transplants, and people taking medications that interfere with immune function (e.g., cytotoxic drugs for the treatment of cancer), among others[5].

Antimicrobial resistance is a major global public health concern and a food safety issue. It can pose a greater human health risk as a result of potential treatment failure, loss of treatment options, and increased likelihood and severity of disease[6]. Today, we are encountering foodborne multidrug-resistant (MDR) microorganisms in clinical and farm settings that are difficult to combat with currently available antibiotics[6][7]. These multiple resistances have been mainly attributed to the proliferation of resistant genes and the ease of dissemination of resistant strains between humans and animals, especially via food of animal origin or fecal contamination[7]. In recent years, attention has been focused on the emergence of therapeutic-antibiotic-resistant strains among the most common foodborne pathogens. These include emerging resistant phenotypes among foodborne pathogens such as *Escherichia coli, Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Listeria monocytogenes,* and *Campylobacter* spp., among others [8]

The susceptibility to a variety of common and opportunistic foodborne infections in HIV-infected people is generally associated with the progressive decline in immunological responses as a result of a low CD4+ T-lymphocyte cell count (Mwambete et al., 2014). Foodborne illnesses caused by drug-resistant pathogens among HIV/AIDS patients are likely to be more serious and last longer. In Zambia, little is

known about the antimicrobial resistance patterns of FBI from HIV/AIDS patients, despite Zambia being one of the countries with a high burden of HIV[9]. This study was therefore set out to determine the antimicrobial resistance patterns of foodborne bacterial isolates from HIV/AIDS patients in Lusaka, Zambia.

Materials and Methods

Study design

This was a cross-sectional study conducted between August and September 2024 to determine the antimicrobial resistance patterns of foodborne isolates from HIV/AIDS patients at the Adult Infectious Disease Clinic, University Teaching Hospital (UTH), Lusaka, Zambia.

Sampling technique

This research used a purposive sampling method to select its participants. This was done after obtaining consent from participants. Participants were selected on purpose based on their knowledge and understanding of the research as well as the criteria for inclusion. All HIV/AIDS patients with one of the complaints of abdominal pain, vomiting, diarrhea, nausea, and fever associated with foodborne contamination who were willing to participate were included. However, all non-HIV/AIDS patients and HIV/AIDS patients who were under antibiotic therapy for two weeks before enrolling in the study were excluded.

Media preparation and sample collection

Media were procured and made from the microbiology unit of the Department of Biological Sciences, University of Zambia, and the Microbiology Laboratory of the University Teaching Hospital. They were prepared using the powder of the following agar media: Salmonella Shigella (SS) agar (HIMEDIA M108-500G), Xylose Lactose Dextrose (XLD) agar (OXOID CM0469B), Mannitol Salt Agar (MSA) (HG000C26.500), Thiosulfate-Citrate-Bile-Salt Sucrose (TCBS) agar (HIMEDIA M189-500G), and Mueller-Hinton agar (HIMEDIA M173-500G). The prepared agar media were quality-controlled for sterility and performance and thereafter kept in a refrigerator for further use[10]. Fresh stool samples for microbiology tests were collected using the rectal swab technique. The swab was inserted beyond the anal sphincter

and rotated so that it carried enough stool sample for culturing[11]. The sample collection was done during the normal routine work of the hospital.

Culture and biochemical identification techniques

Stool samples were streaked onto XLD agar, TCBS agar, and MSA agar plates upon their arrival at the laboratory and were incubated for 24 hours at 37°C. Depending on the results of the XLD agar based on the lactose and non-lactose fermenters, the non-lactose fermenter colonies, which were pink colonies on XLD agar, were further streaked onto Salmonella-Shigella agar for the detection of bacteria of interest such as s*almonella* and *shigella*, according to a previously outlined protocol[10]. Moreover, the isolated bacteria were also prepared on slides using normal saline and heat fixing for Gram staining. Biochemical tests such as LIA (Lysine Iron Agar), SIM (Sulfide Indole Motility), TSI (Triple Sugar Iron), citrate test, and oxidase test were also used for the purpose of biochemical characterization. After the incubation period of 24 hours, results were recorded[12].

Antimicrobial susceptibility testing

Each isolated gram-negative and gram-positive organism was tested against standard antibiotic discs in Mueller Hinton agar. The standard antibiotics used in the current study were ampicillin (10 μg), co-trimoxazole (25 μg), nitrofurantoin (300 μg), chloramphenicol (30 μg), azithromycin (15 μg), amoxicillin-clavulanic acid (30 μg), and oxacillin (1 μg). Antimicrobial susceptibility testing was carried out using the modified Kirby-Bauer agar disc diffusion technique as previously outlined[13] (Mwansa et al., 2013). After the inoculation of test organisms into the Mueller Hinton agar media, the antibiotic discs were placed using a disc dispenser. The plates with the antibiotic discs were incubated at 37°C for 24 hours to observe the zones of inhibition produced by the standard antibiotics. Quality control of antibiotic discs and incubation conditions was ensured according to the laboratory guidelines.[14]

Data analysis

Sociodemographic variables such as age, sex, residence, and data for sample origin were taken from a medical card record from individual participants who were fit for the study. Moreover, the microbiological results obtained from the respective participants were incorporated into the existing data of sociodemographic variables. The data were entered into Microsoft Excel (2016) and were robustly reviewed and cleaned up before further analysis. Finally, the cleaned data were analyzed using SPSS

version 30 (IBM Corp., Armonk, NY, USA) statistical software. Descriptive summaries and data presentations such as percentages/frequency and tables were used. Multinomial logistic regression was used to analyze the MDR and XDR patterns in comparison to the sociodemographic variables. Odds ratio, *p-values*, and confidence intervals were used for checking the statistical significance of the data (a *p-value* < 0.05 was considered statistically significant). Model fitting such as goodness of fit and pseudo-R-square was used for the analysis of multinomial logistic regression.

Ethical Considerations

Ethical approval to conduct the study was obtained from the Natural Science Research and Ethical Committee (NASREC) and the University of Zambia Biomedical Research and Ethical Committee (UNZABREC). Approval from the University of Zambia School of Natural Sciences, the National Health Research Authority (NHRA), and the University Teaching Hospital (UTH) was also obtained. Further, informed consent statements for adults in English and Nyanja were prepared and given to all study participants. To maintain confidentiality, study participants were allocated study-specific codes, and the study data were kept strictly confidential. Furthermore, all methods were carried out in accordance with relevant guidelines and regulations of NARSEC, UNZABREC, and the University of Zambia.

Results

Isolation and identification of bacterial isolates

A total of 54 stool samples were collected from the study participants and processed for identifying foodborne bacteria, and a total of 77 bacteria were isolated, with *Escherichia coli* found to be the most prevalent bacterium with 21 isolates (27.3%), followed by *Proteus vulgaris* with 12 isolates (15.6%), *Staphylococcus aureus* with 11 isolates (14.3%), and *Enterobacter* spp. with 10 isolates (12.3%). However, *Acinetobacter* spp., *Salmonella paratyphi, Aeromonas* spp., and *Pseudomonas aeruginosa* each comprised only 1.3%. The total frequency of the isolated bacteria is shown in Figure 1, and the frequency of the major foodborne pathogens is described in Table 1. *Escherichia coli* was the most prevalent bacterium, followed by *Staphylococcus aureus* and *Shigella* spp.

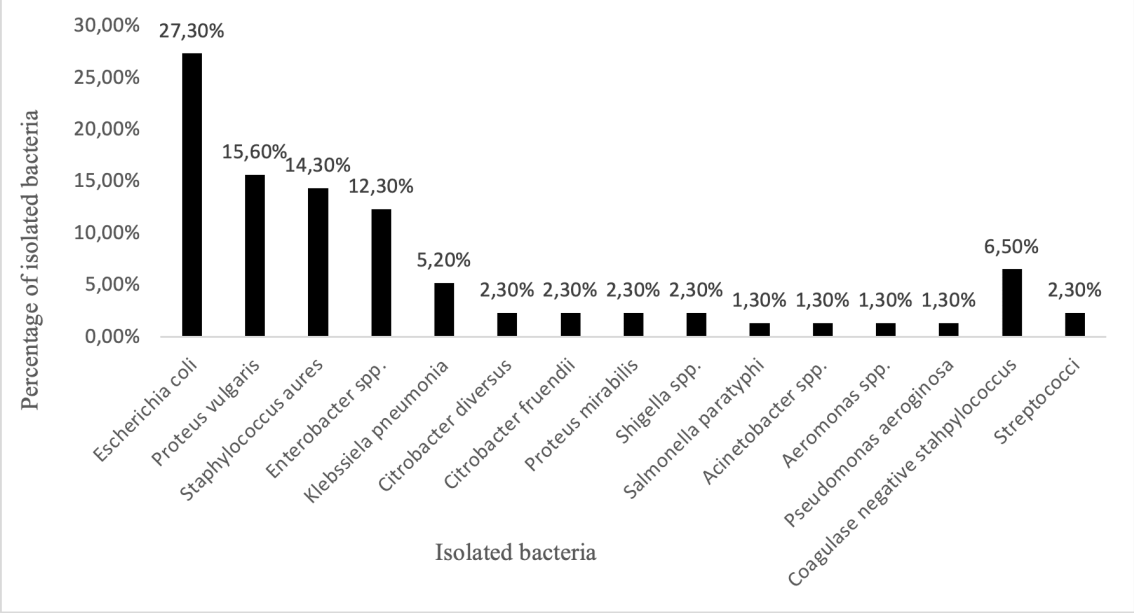

Figure 1. *Overall frequency of isolated bacteria. E. coli* was found to be highly prevalent with 27.30%, followed by *P. vulgaris* at 15.60% and *S. aureus* at 14.30%.

Foodborne bacteria	Frequency	Prevalence
Escherichia coli	21	27.27%
Staphylococcus aureus	11	14.28%
Shigella spp.	2	2.60%
Salmonella paratyphi	1	1.30%
Aeromonas spp.	1	1.30%
Total foodborne bacteria	**36**	**46.75%**
Other enteric bacteria	41	53.25%

Table 1. Frequency of isolated major foodborne bacteria

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Antimicrobial susceptibility test

Escherichia coli was found to be highly resistant to ampicillin (20 resistant isolates, 95.24%) and sulfamethoxazole/trimethoprim (17 resistant isolates, 80.95%) and moderately resistant to azithromycin (9 resistant isolates, 42.85%), amoxicillin/clavulanic acid (8 resistant isolates, 38.09%), and nitrofurantoin (8 resistant isolates, 38.09%). In contrast, *Escherichia coli* was found to be mainly sensitive to chloramphenicol (15 sensitive isolates, 71.43%). The *Salmonella paratyphi* isolate was also highly resistant to ampicillin (100%), sulfamethoxazole/trimethoprim (100%), and amoxicillin/clavulanic acid (100%), but it was also found to be 100% sensitive to chloramphenicol and nitrofurantoin. The same was true for *Shigella* spp., which were resistant to ampicillin, sulfamethoxazole/trimethoprim, and nitrofurantoin (50%). Results are shown in Table 2 below.

Antimicrobial categories	Antimicrobials	*Escherichia coli* (21)			*Shigella* spp. (2)			*Salmonella paratyphi* (1)		
		R	I	S	R	I	S	R	I	S
Penicillins	AMP	20 (95.24%)	1 (4.76%)	0 (0%)	1 (50%)	0 (0%)	1 (50%)	1 (100%)	0 (0%)	0 (0%)
Folate pathway inhibitor	SXT	17 (80.95%)	1 (4.77%)	3 (14.28%)	1 (50%)	0 (0%)	1 (50%)	1 (100%)	0 (0%)	0 (0%)
Macrolide	AZT	9 (42.85%)	7 (33.34%)	5 (23.81%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Beta-lactamase inhibitor	AMC	8 (38.09%)	9 (42.86%)	4 (19.05%)	0 (0%)	2 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Amphenicol	C	4 (19.05%)	2 (9.52%)	15 (71.43%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)
Nitrofuran	F	8 (38.09%)	4 (19.05%)	9 (42.86%)	1 (50%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)

Table 2. Antibiotic susceptibility patterns of foodborne bacteria *Escherichia coli, Shigella* spp., *and Salmonella paratyphi* isolated from stool samples.

Abbreviations: AMP- Ampicillin; SXT-Sulfamethoxazole/trimethoprim; AZT- Azithromycin; AMC-Amoxicillin/clavulanic acid; C-Chloramphenicol; NIT-Nitrofurantoin

Aeromonas spp. were 100% resistant to ampicillin; sulfamethoxazole/trimethoprim and chloramphenicol were found to be 100% sensitive to azithromycin and nitrofurantoin. One of the prevalently isolated foodborne bacteria, *Staphylococcus aureus*, was also among the highly resistant bacteria. It was found to be 100% resistant to azithromycin (11 resistant isolates) and 90.9% resistant to oxacillin (10 resistant isolates), and it was highly sensitive to chloramphenicol (72.72%, 8 sensitive isolates), as shown in Table 3 below.

Antimicrobial categories	Antimicrobials	*Aeromonas spp. (1)*			*Staphylococcus aureus (11)*		
		R	**I**	**S**	**R**	**I**	**S**
Penicillins	AMP	1(100%)	0(0%)	0(0%)	6(54%)	0(0.0%)	5(46%)
	OX	-	-	-	10(90.9%)	1(9.1%)	0(0.0%)
Folate pathway inhibitor	SXT	1(100%)	0(0%)	0(0%)	-	-	-
Macrolide	AZT	0(0%)	0(0%)	1(100%)	11(100%)	0(0.0%)	0(0.0%)
Beta-lactamase inhibitor	AMC	0(0%)	1(100%)	0(0%)	-	-	-
Amphenicol	C	1(100%)	0(0%)	0(0%)	0(0.0%)	3(27.27)	8(72.72%)
Nitrofuran	F	0(0%)	0(0%)	1(100%)	-	-	-

Table 3. Antibiotic susceptibility patterns of *Aeromonas* spp. and *Staphylococcus aureus*

Abbreviations: AMP- Ampicillin; SXT-Sulfamethoxazole/trimethoprim; AZT- Azithromycin; AMC-Amoxicillin/clavulanic acid, C-Chloramphenicol; NIT-Nitrofurantoin, OX-Oxacillin

Distribution of foodborne bacterial isolates according to participant's residence

Foodborne bacterial isolates were tracked to determine which areas have a higher prevalence. This was done by plotting the residences of study participants against the respective foodborne isolates. Figure 2 shows the overall percentage distribution using a stacked bar chart. It was noticed that *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella paratyphi* are prevalent in Bauleni. *Shigella* spp. are common in John Lenge and Matero, whereas *Aeromonas* spp. was found to be common in Kasupe.

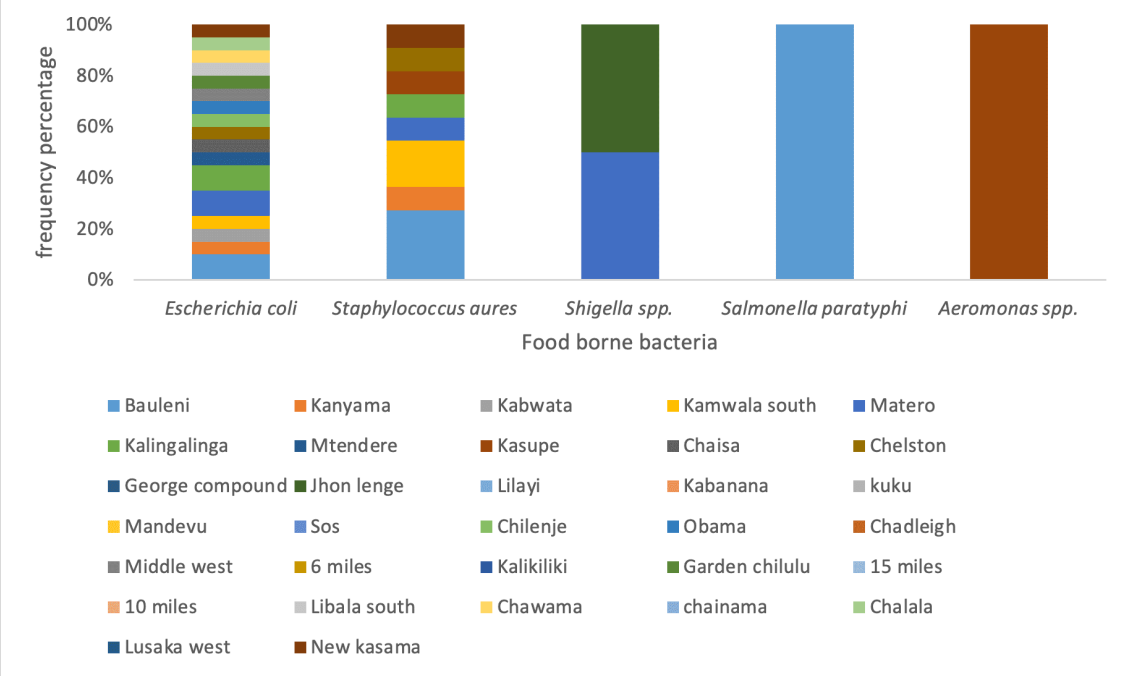

Figure 2. Percentage distribution of foodborne bacteria in different areas of Lusaka and Central province, Zambia. The figure shows the frequency of foodborne bacteria in different places; different colors show places where *S. paratyphi* was highlighted in Bauleni only and *Aeromonas* spp. in Kasupe only.

Multinomial logistic regression model

Most of the multidrug-resistant foodborne bacteria were isolated from females, accounting for 23.53%, from the age group 55 and above (42.9%), and from samples from wards (35.3%). This study has revealed that sample origin was significantly associated with MDR foodborne bacteria (*p-value*= 0.007). The results are shown in Table 4 below.

Variables	Categories	Total	Negative (19) (52.8%)	MDR (7) (19.4%)	XDR (10) (27.8%)	Odds ratio	CI (95%)	*p-value*				
Sex	Male	19	11 (57.9%)	3 (15.80%)	5 (26.3%)	0.637	0.07 - 5.845	0.690				
	Female	17	8 (47.06%)	4 (23.53%)	5 (29.41%)							
Age	25-34	7	3(42.9%)	2(28.6%)	2(28.5%)	0.897	0.295 - 2.725	0.848				
	35-44	10	6 (60%)	0 (%)	4 (40%)							
	45-54	12	6 (50%)	2 (16.7%)	4 (33.3%)							
	55 and above	7	4 (57.1%)	3 (42.9%)	0 (0%)							
Sample origin	AIDC (Outpatient)	19	16 (84.21%)	1 (5.26%)	2(10.53%)	**0.033**	**0.003 - 0.931**	**0.007**				
	Wards (Inpatient)	17	3 (17.65%)	6 (35.3%)	8 (47.05%)							

Table 4. Distribution of MDR Foodborne bacterial isolates

Discussion

This study identified about 15 different bacterial species, where the major foodborne bacterial pathogens comprised 46.75% of the total isolates (in total, 77 bacterial stool isolates were identified). In addition, foodborne bacteria such as *Escherichia coli* and *Staphylococcus aureus* were found to be the most prevalent bacterial isolates, accounting for 27.31% and 14.31%, respectively. *Escherichia coli* has been mentioned as the most prevalent enteric bacterial isolate many times in different studies; for instance, a study by Falodun and his colleagues from Nigeria[15] found *Escherichia coli* in 41.6% of all isolates. Another study by Webale and his colleagues in Kenya showed that 36.4% of the total isolates were comprised of *Escherichia coli*[16]. These results are similar to the current study, making it the most commonly encountered bacterium. *Escherichia coli* is considered normal flora and has been beneficial in synthesizing vitamins B12 and K; however, in immunocompromised individuals such as HIV/AIDS patients, it can cause

infections such as gastrointestinal infections, upper and lower respiratory tract infections, and even bacteremia in adverse conditions[17].

Although studies by Falodun and Webale reported a low prevalence of *Staphylococcus aureus* in stool samples, the current study found a higher prevalence of *Staphylococcus aureus* in stool samples [18]. Generally, *Staphylococcus aureus* is an aerobic bacterium that resides in nasal and skin areas and is not expected to be isolated frequently from stool samples[18]. The main reason for this high amount of isolation from stool samples could be that participants might have eaten foods infested by the bacteria, although a deeper epidemiological investigation is needed[18]. Secondly, nosocomial *Staphylococcus aureus* infections, which are a common scenario in inpatient departments, can contaminate the stool[19]. A study done in the USA showed that intestinal colonization *by Staphylococcus aureus* among hospitalized patients has been associated with an increased risk of staphylococcal infection and could potentially contribute to transmission[20]. In comparison to nares colonization only, nares and intestinal colonization was associated with an increased frequency of positive skin cultures (41% versus 77%; p = 0.001) and trends toward increased environmental contamination (45% versus 62%; p = 0.188) and acquisition on investigators' hands (36% versus 60%; p = 0.057)[20].

Different antibiotics from different antibiotic classes have been used to follow and study the patterns of antimicrobial resistance of isolated foodborne bacteria. The resistance patterns of *Escherichia coli* to ampicillin and sulfamethoxazole/trimethoprim in the current study are higher compared to a study in Kenya[16]; the reasons for the high prevalence could be due to the prolonged use of these antibiotics along with ARV drugs, which are recommended as a guideline for HIV/AIDS. On the contrary, it was lower when comparing resistance to chloramphenicol. The reason for this could be the low prescription rate of this antibiotic by clinicians due to its toxic effect. Another study in Zambia[21] has shown similar results in resistance to sulfamethoxazole/trimethoprim, which is 90.2%, but higher sensitivity to amoxicillin/clavulanic acid, with 98.2%. In contrast, another study in Cameroon showed similar results in every aspect in that there was 33% resistance to amoxicillin/clavulanic acid and 83.3% sensitivity to chloramphenicol [16]. Overall, higher resistance to ampicillin and sulfamethoxazole/trimethoprim and higher sensitivity to chloramphenicol were noticed in most of the literature except by Webale and his colleagues[16].

Similarly, *Salmonella paratyphi* isolate recorded a very high resistance to amoxicillin/clavulanic acid compared to a study in Cameroon that showed 40% resistance to amoxicillin/clavulanic acid[14]. The

same study showed a 20% resistance to chloramphenicol, while it was 100% sensitive to chloramphenicol in the current study. The same is true with *Shigella* spp., which were resistant to ampicillin, sulfamethoxazole/trimethoprim, and nitrofurantoin (50%). Moreover, *Aeromonas* spp. were 100% resistant to ampicillin, sulfamethoxazole/trimethoprim, and chloramphenicol and were found to be 100% sensitive to azithromycin and nitrofurantoin. The use of unprescribed antibiotics is one of the reasons that leads to antimicrobial resistance and is a common problem in sub-Saharan African countries[22]. For that reason, most of the isolates are resistant to ampicillin and sulfamethoxazole/trimethoprim and moderately sensitive to chloramphenicol and nitrofurantoin.

Staphylococcus aureus was also among the highly resistant bacteria. It was found to be 100% resistant to azithromycin and 90.9% resistant to oxacillin, and it was highly sensitive to chloramphenicol, with 72.72%. A study by Kates and his colleagues has shown that resistance to oxacillin is seen in 43.1% of stool *Staphylococcus aureus* isolates, which is lower than the resistance to oxacillin recorded by the current study (90.9%)[23]. In a study in Vietnam, similar results have been recorded as in the current study, implicating high resistance to azithromycin up to 82.28% and oxacillin up to 70%[24]. The other angle this study tried to see in accordance with patterns of antimicrobial resistance is the incidence of multidrug resistance (MDR) and extensive drug resistance (XDR). Hence, a high incidence was noticed, accounting for 19.4% of MDR cases and 27.8% of XDR cases. In other words, those standard antibiotics failed to treat infections from approximately 48.2% of the total isolated foodborne bacteria. HIV/AIDS patients, especially inpatients, are under treatment with different antibiotics for different reasons, such as respiratory infections, urinary tract infections, gastrointestinal infections, and other infections. Inappropriate use of antibiotics has also been linked to such patients.

The current study has shown that MDR cases are mainly present in inpatient participants compared to outpatient participants (p-value 0.007). A similar study conducted in Iran showed that inpatient resistance was higher than outpatient resistance[25]. This could be due to excessive exposure to antibiotics, either prescribed but not strictly taken or taking unprescribed antibiotics. The use of chloramphenicol, which was sensitive to the majority of the foodborne bacteria (29.04%) in inpatients, is advised according to the current study. Most of the participants resided in Bauleni (with 12.96%) and Kanyama (with 7.4%). Highly populated areas are a common source of bacterial infections throughout the world, especially for foodborne and waterborne illnesses (Kim and Ahn, 2022). This is because of the poor sanitation system, poor individual and community-based hygienic practices, and easy propagation of

those bacterial agents from person to person via food and water in accordance with population density[10].

The study recommends conducting molecular studies of antimicrobial resistance patterns of foodborne bacterial isolates, as this will give a better understanding of the resistance mechanism. It is also recommended that future studies incorporate both clinical samples and food samples for analysis to better understand to what extent foodborne bacteria propagate. This will give a deeper concept of the 'One Health approach to antimicrobial resistance'. Moreover, sequencing of *Staphylococcus aureus*, a nosocomial infection in inpatient wards, for a possible outbreak analysis are highly recommended angles that future studies should focus on.

Conclusion

Results have shown that the resistance patterns of the isolates are at an alarming rate. This indicates that the issue of antimicrobial resistance is now heading towards a dangerous phase, where multidrug-resistant foodborne isolates are imposing a great burden on public health. AMR affects every person on the planet, but more severe effects have been seen in people living with HIV/AIDS. The lower immunity seen in people living with HIV/AIDS allows such bacteria to create unfavorable conditions for treating infections, leading to possible death. The study showed that a promising antibiotic, chloramphenicol, is still a viable option. The antibiotic was found to be effective in most cases for both Gram-positive and Gram-negative foodborne bacterial isolates. However, better handling in prescribing this antibiotic is a timely issue to discuss. MDR and XDR cases were seen in high numbers, which makes the study strongly address where and how AMR of foodborne pathogens, stated as "a silent pandemic" by the WHO, is heading. Therefore, effective surveillance and management from stakeholders (government bodies, health, and agricultural sectors) in investigating and controlling AMR is of great concern.

Statements and Declarations

Funding

This study was not funded by any agency.

Conflicts of Interest

The authors declare no competing interests.

Ethical Approval

Ethical approval was obtained, and consent was obtained from both the hospital management and patients to collect samples and data.

Data Availability

Data are available from the corresponding author and can be made available upon request.

Author contribution

ARM reviewed sample collection, laboratory and data analysis, and drafted the manuscript; JM analyzed the data; MZ and NM drafted the manuscript; SM conceptualized the study and drafted the manuscript.

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