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Antimicrobial resistance patterns of enteric bacteria in HIV sero-positive patients: A regional hospital-based cross-sectional survey in Kumasi metropolis, Ghana

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Abstract:

Background: Enteric bacterial infections are at least ten times more common in HIV individuals compared to the general population. This results in frequent consumption of antibiotics either for prophylactic or treatment purposes, resulting in a rise in emergence of multidrug-resistant (MDR) bacteria. The paucity of research on antibiotic resistance in enteric bacteria among HIV individuals in secondary and primary healthcare settings in Ghana necessitated this study, which investigated the antibiotic susceptibility and resistance patterns of enteric bacterial isolates from HIV-positive individuals at three healthcare facilities in Kumasi metropolis, Ghana.
Methodology: This was a descriptive cross-sectional survey of 57 HIV-seropositive individuals on anti-retroviral therapy at three selected district hospitals in Kumasi metropolis, for resistance pattern of enteric bacteria. Faecal samples from HIV-positive individuals were collected for microbiological analysis, using differential and selective agar media and conventional biochemical assays for isolation, preliminary characterisation and presumptive identification of bacterial isolates. Confirmatory identification of the isolates was performed using MALDI-TOF MS. Antimicrobial susceptibility testing by the Kirby-Bauer disk diffusion method was used to evaluate the susceptibility of the isolates to a panel of 10 antimicrobial agents. Data were analysed with WHONET software, GraphPad Prism, Microsoft Excel and SPSS. Association of demographic data and other patients' parameters was determined using Chi-square tests with 95% confidence interval and $p<0.05$ was considered significant.
Results: Thirty enteric bacterial isolates were confirmed from the 57 HIV-seropositive individuals, comprising predominantly *Escherichia coli* (80.0%), *Klebsiella pneumoniae* (10.0%), *Enterococcus faecalis* (6.7%), and *Enterobacter cloacae* (3.3%). Antimicrobial susceptibility testing showed resistance rates ranging from 3.3% to 100.0%, with majority of isolates susceptible to ciprofloxacin and meropenem. Sixty per cent of the isolates were MDR and *E. coli* and *K. pneumoniae* isolates were highly resistant to amoxicillin-clavulanic acid and ampicillin.
Conclusion: *E. coli* was the most frequent enteric bacterial isolate carried by HIV-infected individuals in this study. Over 60% of the enteric bacteria were multidrug-resistant. Meropenem and ciprofloxacin were the antibiotics with high *invitro* inhibitory activity against the isolates. The use of these antibiotics for therapy should however be guarded to avoid rapid resistance development by bacterial pathogens to these agents.

Keywords: HIV seropositive. Enterobacteria, *E. coli,* meropenem, ciprofloxacin

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Profils de résistance aux antimicrobiens des bactéries entériques chez les patients séropositifs pour le VIH: une enquête transversale régionale menée en milieu hospitalier dans la métropole de Kumasi, au Ghana

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Résumé:

Contexte: Les infections bactériennes entériques sont au moins dix fois plus fréquentes chez les personnes infectées par le VIH que dans la population générale. Cela entraîne une consommation fréquente d'antibiotiques, que ce soit à titre prophylactique ou thérapeutique, et une augmentation de l'émergence de bactéries multirésistantes (MR). Le manque de recherches sur la résistance aux antibiotiques des bactéries entériques chez les personnes infectées par le VIH dans les établissements de soins de santé primaires et secondaires au Ghana a rendu nécessaire la réalisation de cette étude. Cette étude a examiné la sensibilité aux antibiotiques et les profils de résistance d'isolats de bactéries entériques provenant de personnes infectées par le VIH dans trois établissements de santé de la métropole de Kumasi, au Ghana.
Méthodologie: Il s'agissait d'une enquête transversale descriptive menée auprès de 57 personnes séropositives sous traitement antirétroviral dans trois hôpitaux de district sélectionnés de la métropole de Kumasi, afin d'évaluer le profil de résistance des bactéries entériques. Des échantillons de selles de personnes séropositives ont été prélevés pour analyse microbiologique, à l'aide de milieux gélosés différentiels et sélectifs, ainsi que de dosages biochimiques conventionnels pour l'isolement, la caractérisation préliminaire et l'identification présomptive des isolats bactériens. L'identification confirmatoire des isolats a été réalisée par spectrométrie de masse MALDI-TOF. Des tests de sensibilité aux antimicrobiens par diffusion sur disque de Kirby-Bauer ont été utilisés pour évaluer la sensibilité des isolats à un panel de 10 agents antimicrobiens. Les données ont été analysées avec les logiciels WHONET, GraphPad Prism, Microsoft Excel et SPSS. L'association des données démographiques et des autres paramètres des patients a été déterminée par des tests du Chi carré avec un intervalle de confiance à 95% et une valeur de $p<0,05$ a été considérée comme significative.
Résultats: Trente isolats de bactéries entériques ont été confirmés chez 57 personnes séropositives pour le VIH, comprenant principalement *Escherichia coli* (80,0%), *Klebsiella pneumoniae* (10,0%), *Enterococcus faecalis* (6,7%) et *Enterobacter cloacae* (3,3%). Les tests de sensibilité aux antimicrobiens ont montré des taux de résistance allant de 3,3% à 100,0%, la majorité des isolats étant sensibles à la ciprofloxacine et au méropénème. Soixante pour cent des isolats étaient multirésistants, et les isolats d'*E. coli* et de *K. pneumoniae* étaient hautement résistants à l'amoxicilline-acide clavulanique et à l'ampicilline.
Conclusion: E. coli était l'isolat de bactérie entérique le plus fréquemment présent chez les personnes infectées par le VIH dans cette étude. Plus de 60 % des bactéries entériques étaient multirésistantes. Le méropénème et la ciprofloxacine étaient les antibiotiques ayant une forte activité inhibitrice in vitro contre les isolats. L'utilisation thérapeutique de ces antibiotiques doit toutefois être prudente afin d'éviter le développement rapide d'une résistance des bactéries pathogènes à ces agents.

Mots-clés: Séropositivité au VIH, entérobactéries, *E. coli*, méropénème, ciprofloxacine

Introduction:

HIV/AIDS remains a disease that weakens the host defences against many infections as it affects the immune system even in the face of improved therapy (1). The World Health Organisation (WHO) indicates that over 39 million (33.1–45.7) people were living with HIV at the end of 2022 (2). The Ghana national HIV and AIDS strategic plan (2021-2025) estimates 346,120 (91.71% adults and 8.29% children) people living with HIV (PLHIV), with an adult national HIV prevalence of 1.68%. In Ghana, the Ashanti region is estimated to have an HIV prevalence of 1.8% (3).

Due to the immunocompromised nature of PLHIV, they are at risk of frequent antibiotic consumption, either for treatment or prophylaxis of infections (4). Overuse and misuse of these antibiotics can result in the development of different resistance mechanisms by enteric bacteria (5,6). Unfortunately, HIV-infected individuals may harbour resistant bacterial strains because of their frequent exposure to antibiotic medication and prophylaxis (7).

The current management of bacterial infections could be difficult owing to multidrug-resistant (MDR) bacteria and limited treatment options (8). Some enteric bacterial pathogens, such as *Salmonella* spp., *Klebsiella* spp., *Campylobacter* spp., *Shigella* spp., *Clostridioides difficile* and different strains of *Escherichia coli* have been identified as causative agents of diarrhoea in HIV-infected patients with the potential to induce severe diarrhoeal disease when they are multidrug-resistant (9).

Enteric bacterial infections are at least ten times more common in HIV patients than in the general population (10). Enteric bacterial pathogens with high antibiotic resistance have been reported among PLHIV (11), however such reports are few and unavailable especially in low-and-middle income countries, both globally and in sub-Saharan Africa (10).

Surveillance aids in the identification of factors that contribute to the spread of antimicrobial resistance (AMR) in specific areas, allowing for the implementation of control measures, however, most investigations on the extent of antibiotic resistance in enteric bacteria from immunocompromised individuals are carried out in tertiary-level health institutions, without recourse to the primary and secondary levels, which are typically the initial place of visit for the treatment of most enteric infections.

Efforts to reduce enteric pathogen transmission cannot be overemphasised, however, the rate of resistance of diverse infectious agents

varies by geographical area and country (4). This study south to investigate the antibiotic resistance situation in enteric bacteria from HIV-infected patients from secondary-level health institutions in the Ashanti region of Ghana.

Materials and method:

Study setting and design:

This was a descriptive cross-sectional study conducted at three hospitals; Suntreso Hospital, Manhyia Hospital and Kumasi South Hospital, in Kumasi metropolis, Ghana. The three district hospitals serve as primary health centres for over 70% of the inhabitants in the Kumasi metropolis.

Ethical approval:

The study was approved by the Research and Development Unit of the Komfo Anokye Teaching Hospital (RD/CR20/095). Consent from the various hospitals was granted, and verbal and written consent was obtained from the study participants.

Study population, participants and sample size:

The study included HIV/AIDS patients who were visiting the hospitals for routine check and fill-up of their Highly Active Anti-retroviral Therapy (HAART) medications. To be eligible for inclusion in the study, participants should not have been on or received any specific antibiotic therapy in the previous two weeks prior to the study. Diabetic patients, pregnant women, smokers and patients on oestrogen were also exempted from the study.

The sample size was determined using the formulae (12); $(Z_{1-\alpha/2})^2 \times p\ (1-p)/d^2$, where $Z_{1-\alpha/2}$ is the standard normal variate at 5% (p<0.05 =1.96), p is prevalence of PLHIV (2% or 0.02) in the Ashanti region (13), and d is margin of error of 5% (0.05). This gave a calculated sample size of 30 HIV/AIDS patients, but 57 patients were recruited into the study.

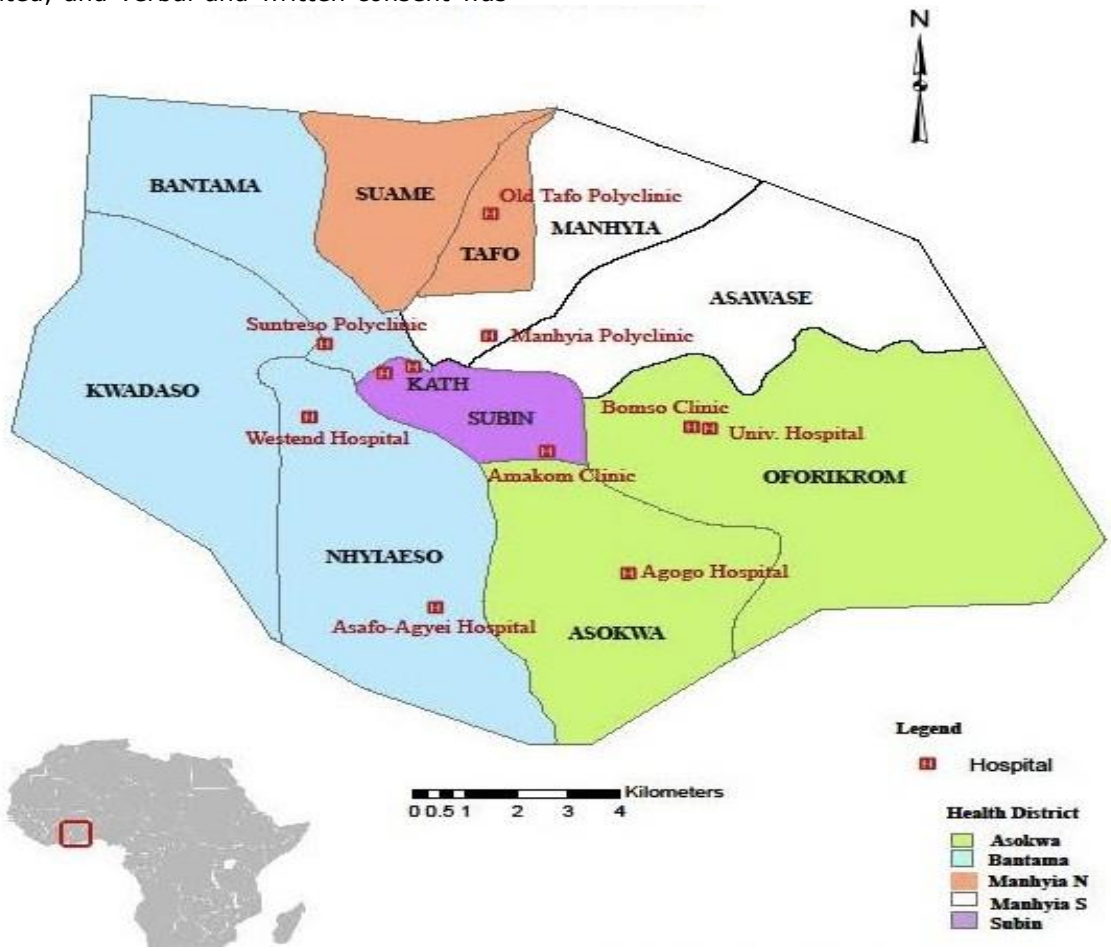

Fig 1: Demographical representation of the Kumasi metropolis health district and health facilities (http://mci.ei.columbia.edu/millennium-cities/kumasi-ghana/kumasi-maps-and-population-data/)

Data collection:

Participants were given a consent form to sign, after the research had been explained and they agreeing to submit their stool samples for testing and giving consent to access personal data from their hospital records. The personal data accessed included their name, age, sex, contact details, medication history (duration of HAART treatment) and current medication.

Sample collection, bacterial isolation and preliminary identification:

Stool samples were collected from participants using sterile disinfectant-free containers. The specimens were sent to the labo ratory, emulsified with sterile water and cultured on Xylose Lysine Deoxycholate agar (XLD agar), MacConkey agar and Mannitol Salt Agar plates, and incubated at 37°C for 24 h. The isolated organisms were preliminarily identified based on colonial morphology, Gram stain, and conventional biochemical tests, and confirmed by Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (14).

Confirmation of isolates using MALDI-TOF:

MALDI-TOF of ribosomal and housekeeping proteins was used to confirm the isolates. Preliminarily identified bacteria isolates were cultured on tryptone soya agar, a colony was picked from a cultured plate and spotted (forming a thin film layer of the colony) on a MALDI-TOF MS target plate. The cells were then treated with formic acid (Surechem, Needham, UK) on the target plate on the MALDI Biotyper (Bruker Daltonic GmbH). Generated spectra were compared with current libraries (MALDI Flex Control software system Server Version: 4.1.31 and 60 Hz Nitrogen Laser (337 nm wavelength) and log scores generated (Scores of the log were classified as: > 2.0 = High confidence identification, 1.7–2.0 = Low confidence and < 1.7.0 = Unreliable).

Antimicrobial susceptibility testing:

Antibiotic susceptibility tests on the confirmed isolates were done by the Kirby–Bauer method. The antibiotics tested included amikacin (AMK 30µg), amoxicillin/clavulanic acid (AMC 20/10µg), ampicillin (AMP 10µg), aztreonam (ATM 30µg), cefotaxime (CTX 30µg), ceftriaxone (CRO 30µg), cefuroxime (CXM 30µg), chloramphenicol (CHL 30µg), ciprofloxacin (CIP 5µg), gentamicin (CN 30 µg), meropenem (MEM 10µg), and tetracycline (TEN 30µg).

Colonies of the bacterial isolates were suspended in 3ml nutrient broth and incubated for 24 hours. The turbidity of the suspension of the isolate was standardised by adjusting its turbidity to match 0.5 McFarland standard (Hardy Diagnostics, Santa Maria, USA), which is equivalent to approximately 1.5×10^8 colony forming units (CFU) per millilitre (15). A sterile cotton swab was dipped into the standardised bacteria suspension and swabbed evenly across the entire surface of Mueller Hinton (MH) agar plate to obtain a semiconfluent growth. The plate was incubated aerobically at 37°C for 24 hours.

After incubation, clear zones around the antibiotic discs (zones of inhibition) were measured; taking the average of the horizontal diameter, vertical diameter and diagonal diameter of the zones and interpreted as sensitive, intermediate or resistant based on the breakpoint criteria of the Clinical and Laboratory Standards Institute (16). Isolates showing resistance to three or more categories of antibiotics were considered as multidrug-resistant bacteria (17).

Analysis of data:

All data obtained were stored on a secured hard drive at the Department of Pharmaceutics, KNUST. WHONET software, GraphPad Prism from GraphPad Software Inc., USA, version 8.0 and Microsoft Excel from Microsoft Office Professional Plus 2016, USA, were used to analyse the data obtained. WHONET software was used to determine the sensitivity profile of isolates using zone breakpoints from 2024 CLSI. A statistical association of demographic data and other parameters was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics V. 30) with Chi-square tests and 95% confidence interval, and $p<0.05$ considered significant.

Results:

Sources of samples and prevalence of enteric bacteria carriage among the participants:

Stool samples were obtained from 57 HIV-seropositive patients in the 3 hospitals (Suntreso Hospital, Kumasi South Hospital and Manhyia Hospital) within the Kumasi metropolis of Ghana. Forty (40) (70.2%) were from Suntreso Hospital, 11 (19.3%) from Manhyia Hospital and 6 (10.5%) from Kumasi South Hospital (Table 1).

Table 1: Sources of samples and prevalence of enteric bacteria carriage among HIV-seropositive patients

Facility	HIV- seropositive samples	Samples positive for enteric bacteria	x^2	*p* value
Suntreso	40 (70.2)	15 (37.5)	12.832	0.0016*
Manhyia	11 (19.3)	9 (81.8)		
Kumasi	6 (10.5)	6 (100.0)		
Total	57 (100.0)	30 (52.6)		

x^2 = Chi square; * = statistically significant

Table 2: Sociodemographic characteristics and medication history of the HIV-seropositive participants

Parameters	Number	Percentage (%)
Sex		
Female	48	84.2
Male	9	15.8
Age group (years)		
0-20	1	1.8
20-40	23	40.4
40-60	25	43.9
> 60	8	14.0
Duration of therapy (months)		
0-24	20	35.1
24-48	13	22.8
48-72	10	17.5
72-96	6	10.5
96-120	4	7.0
120-144	3	5.3
144-168	1	1.8
Medication history		
3TC/TDF/DTG	40	70.2
3TC/TDF/EFV	14	24.6
3TC/ZDV/EFV	1	1.8
ABC/3TC/LPV/r	1	1.6
FTC/TDF/EFV	1	1.6
Total	**57**	**100.0**

3TC – Lamivudine, TDF – Tenofovir, DTG – Dolutegravir, EFV – Efavirenz, ZDV – Zidovudine, LPV/r – Lopinavir/Ritonavir, FTC – Emtricitabine

Sociodemographic characteristics and medication history of the participants

Forty-eight (84.2%) of the samples were obtained from females while 9 (15.8%) samples were from males (Table 2). One (1.75%) sample was obtained from a patient aged 0-20 years, 23 (40.4 %) between 20-40 years, 25 (43.9 %) between 40-60 years and 8 (14.0%) above 60 years. A total of 20 participants (35.1%) have been on HAARTs for 0 to 24 months, with just one person having been on HAARTs for about 156 months (13 years). The most common HAART combination used was Lamivudine/Tenofovir/Dolutegravir (used by 70.2 % of the participants).

Frequency distribution of confirmed enteric bacterial isolates:

Thirty (30) bacterial isolates were identified and confirmed from the samples based on the culture media, biochemical reactions and MALDI-TOF (Table 3), giving enteric bacteria carriage rate of 52.6% in the study population. Fifteen (37.5%) isolates were obtained from Suntreso Hospital (SH), 9 (81.8%) from Manhyia Hospital (MH), and 6 (100.0%) from Kumasi South Hospital (KH) (x^2=12.832, p=0.0016) (Table 1).

Twenty-four (80.0%) of the isolates were *Escherichia coli*, 3 (10.0%) *Klebsiella pneumoniae*, 2 (6.7%) *Enterococcus faecalis*, and 1 (3.3%) *Enterobacter cloacae* isolate (Table 4). From the 15 isolates obtained from SH, 13 (86.7%) were *E. coli*, and 1 (6.7%) each were *E. faecalis* and *K. pneumoniae.* From the 9 isolates obtained from MH, 6 (66.7%) were *E. coli*, 2 (18.2%) were *K. pneumoniae* and 1 (9.1%) was *E. cloacae*. From the 6 isolates obtained from KH, 5 (83.3%) were *E. coli*, and 1 (16.7%) was *E. faecalis* (Table 4).

Table 3: MALDI-TOF confirmatory identification lists of the microbial isolates

Sample Number	Organism (First match)	Score value	Organism (second match)	Score value
1.	*Escherichia coli*	2.41	*Escherichia coli*	2.26
2.	*Escherichia coli*	2.28	*Escherichia coli*	2.26
3.	*Enterococcus faecalis*	2.21	*Enterococcus faecalis*	2.21
4.	*Escherichia coli*	1.75	*Escherichia coli*	1.84
5.	*Escherichia coli*	2.40	*Escherichia coli*	2.37
6.	*Enterococcus faecalis*	2.22	*Enterococcus faecalis*	2.19
7.	*Escherichia coli*	2.17	*Escherichia coli*	2.16
8.	*Escherichia coli*	2.42	*Escherichia coli*	2.33
9.	*Escherichia coli*	2.36	*Escherichia coli*	2.35
10.	*Escherichia coli*	2.40	*Escherichia coli*	2.35
11.	*Escherichia coli*	2.35	*Escherichia coli*	2.25
12.	*Escherichia coli*	2.41	*Escherichia coli*	2.37
13.	*Klebsiella pneumoniae*	2.33	*Klebsiella pneumoniae*	2.33
14.	*Escherichia coli*	2.07	*Escherichia coli*	2.07
15.	*Escherichia coli*	1.74	*Escherichia coli*	1.73
16.	*Escherichia coli*	2.01	*Escherichia coli*	1.81
17.	*Klebsiella pneumoniae*	2.21	*Klebsiella pneumoniae*	2.17
18.	*Escherichia coli*	2.21	*Escherichia coli*	2.13
19.	*Escherichia coli*	2.00	*Escherichia coli*	1.86
20.	*Escherichia coli*	2.15	*Escherichia coli*	1.94
21.	*Escherichia coli*	2.12	*Escherichia coli*	2.08
22.	*Escherichia coli*	2.33	*Escherichia coli*	2.30
23.	*Escherichia coli*	2.17	*Escherichia coli*	2.16
24.	*Escherichia coli*	2.32	*Escherichia coli*	2.29
25.	*Escherichia coli*	2.28	*Escherichia coli*	2.22
26.	*Escherichia coli*	2.20	*Escherichia coli*	2.20
27.	*Escherichia coli*	2.29	*Escherichia coli*	2.21
28.	*Escherichia coli*	1.89	*Escherichia coli*	1.80
29.	*Klebsiella pneumoniae*	2.25	*Klebsiella pneumoniae*	2.07
30.	*Enterobacter cloacae*	2.20	*Enterobacter cloacae*	2.10

Table 4: Frequency of enteric bacterial isolates with respect to hospital facility

Bacterial isolate	Number of bacterial isolate (%)			Total (%_
	SH	MH	KH	
Escherichia coli	13 (86.7)	6 (66.7)	5 (83.3)	24 (80.0)
Klebsiella pneumoniae	1 (6.7)	2 (18.2)	0	3 (10.0)
Enterococcus faecalis	1 (6.7)	0	1 (16.7)	2 (6.7)
Enterococcus cloacae	0	1 (9.1)	0	1 (3.4)
Total	**15**	**9**	**6**	**30 (100.0)**

SH: Suntreso Hospital; MH: Manhyia Hospital; KH: Kumasi South Hospital.

Antibiotic resistance profile of isolates:

Ten enteric bacterial isolates (33.3%) were resistant to cefotaxime, 15 (50.0%) to ceftriaxone and 5 (16.7%) to cefuroxime. Two isolates (6.7%) each were resistant to gentamicin and amikacin. Also, 29 (96.7%) and 30 (100.0%) isolates were resistant to ampicillin and amoxicillin-clavulanic acid, respectively. One isolate (3.3%) each was resistant to meropenem and ciprofloxacin. Twelve isolates (40.0%) were resistant to aztreonam, 19 (63.3%) to tetracycline and 3 (10.0%) to chloramphenicol (Fig 2).

Multidrug-resistant isolates:

Thirteen (54.2%) of the 24 *E. coli* isolates were MDR, 7 of these 13 (53.8%) were resistant to 4 antibiotics, 2 (15.4%) to 5 antibiotics, and 4 (30.8%) to 6 antibiotics (Table 6). Eight MDR *E. coli* isolates were from SH, 3 from MH, and 2 from KH (Fig 3). Two *K. pneumoniae* isolates were MDR and were resistant to 4 antibiotics, and all from MH. Two *E. faecalis* isolates were MDR, with 1 isolate from SH resistant to 10 antibiotics, and 1 isolate from KH resistant to 7 antibiotics (Fig 3, Table 6)

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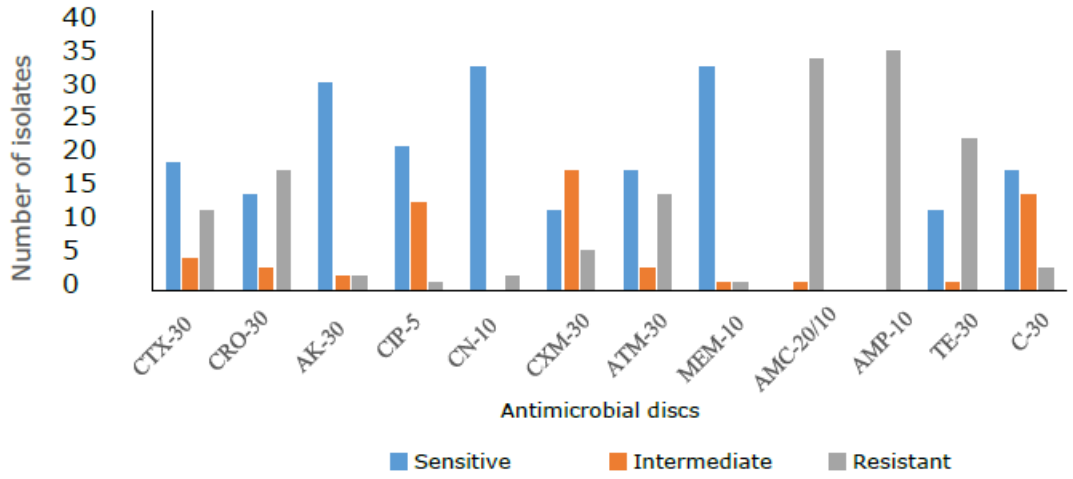

AK-30: Amikacin 30 µg; AMC-20/10: Amoxicillin 20 µg + clavulanic acid 10 µg; AMP-10: Ampicillin 10 µg; ATM-30: Aztreonam 30 µg; CTX-30: Cefotaxime 30 µg; CRO-30: Ceftriaxone 30 µg; CXM-30: Cefuroxime 30 µg; C-30: Chloramphenicol 30 µg; CIP-5: Ciprofloxacin 5 µg; CN-30: Gentamicin 30 µg; MEM-10: Meropenem 10 µg; TE-30: Tetracycline 30 µg

Fig 2: Antibiotic susceptibility and resistance of total bacterial isolates from all samples

Table 5: Antimicrobial resistance of individual bacterial species

Antimicrobial agent	***E. coli* (n=24)**	***K. pneumoniae* (n=3)**	***E. faecalis* (n= 2)**	***E. cloacae* (n= 1)**
CTX-30	6 (25.0)	2 (66.7)	2 (100.0)	0
CRO-30	13 (54.2)	0	2 (100.0)	0
AK-30	0	1 (33.3)	1 (50.0)	0
CIP-5	1 (4.2)	1 (33.3)	0	0
CN-10	0	0	1 (50.0)	0
CXM-30	3 (12.5)	0	2 (100.0)	0
ATM-30	8 (33.3)	1 (33.3)	2 (100.0)	1 (100.0)
MEM-10	0	0	1 (50.0)	0
AMC-20/10	24 (100.0)	3 (100.0)	1 (50.0)	1 (100.0)
AMP-10	24 (100.0)	3 (100.0)	2 (100.0)	1 (100.0)
TE-30	16 (66.7)	0	2 (100.0)	1 (100.0)
C-30	2 (8.3%)	0	1 (50.0)	0

AK-30: Amikacin 30 µg; AMC-20/10: Amoxicillin 20 µg + clavulanic acid 10 µg; AMP-10: Ampicillin 10 µg; ATM-30: Aztreonam 30 µg; CTX-30: Cefotaxime 30 µg; CRO-30: Ceftriaxone 30 µg; CXM-30: Cefuroxime 30 µg; C-30: Chloramphenicol 30 µg; CIP-5: Ciprofloxacin 5 µg; CN-30: Gentamicin 30 µg; MEM-10: Meropenem 10 µg; TE-30: Tetracycline 30 µg

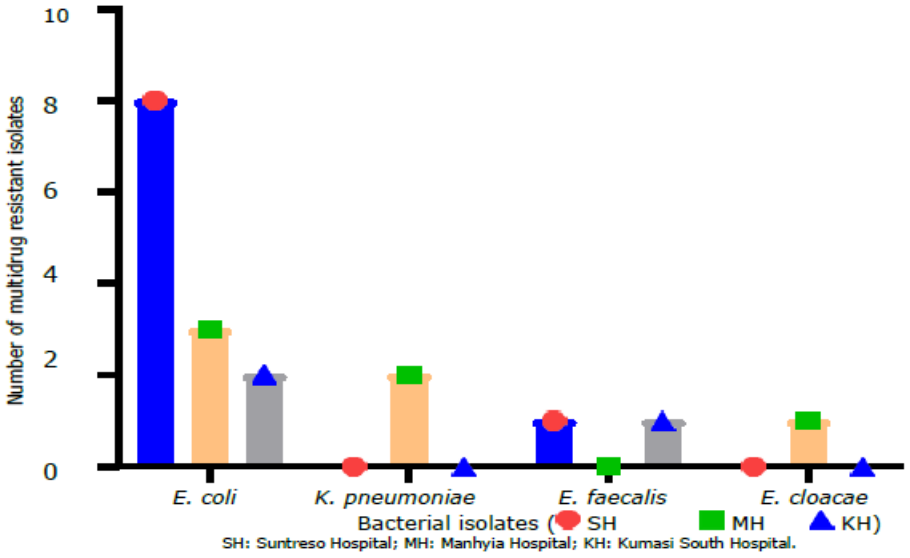

Fig 3: Sources of multidrug-resistant bacterial isolates

Table 6: Multidrug-resistance pattern of enteric bacterial isolates from stool samples of HIV-sero-positive patients from selected hospitals in Kumasi, Ghana

Bacterial isolates and MDR profiles	**Frequency (%)**
***Escherichia coli* (n=24)**	
CRO, AMP, AMC, TE	3 (23.0)
ATM, AMP, AMC, TE	2 (15.4)
AMP, AMC, TE, C	1 (7.7)
CTX, ATM, AMP, AMC	1 (7.7)
CTX, CRO, ATM, AMP, AMC	1 (7.7)
CRO, AMP, AMC, TE, C	1 (7.7)
CTX, CRO, ATM, AMC, AMP, TE	1 (7.7)
CTX, CRO, CXM, ATM, AMC, AMP	3 (23.0)
Total	13 (54.2)
***Klebsiella pneumoniae* (n=3)**	
CTX, ATM, AMP, AMC	1 (50.0)
CRO, CIP, AMP, AMC, TE	1 (50.0)
Total	2 (67.7)
***Enterococcus faecalis* (n=2)**	
CTX, CRO, CXM, ATM, AMC, AMP, TE	1 (50%)
CTX, CRO, AK, CN, CXM, ATM, MEM, AMP, TE, C	1 (50%)
Total	2 (100.0)

AK-30: Amikacin 30 µg; AMC-20/10: Amoxicillin 20 µg + clavulanic acid 10 µg; AMP-10: Ampicillin 10 µg; ATM-30: Aztreonam 30 µg; CTX-30: Cefotaxime 30 µg; CRO-30: Ceftriaxone 30 µg; CXM-30: Cefuroxime 30 µg; C-30: Chloramphenicol 30 µg; CIP-5: Ciprofloxacin 5 µg; CN-30: Gentamicin 30 µg; MEM-10: Meropenem 10 µg; TE-30: Tetracycline 30 µg; SH: Suntreso Hospital; MH: Manhyia Hospital; KH: Kumasi South Hospital

Statistical analysis of sociodemographic data and resistance patterns:

The analysis shows that there was significant association between isolate resistance to amikacin, cefuroxime and ciprofloxacin, with respect to age, current medication and duration of therapy ($p<0.05$) (Tables 7).

All isolates were susceptible to meropenem and tetracycline and resistant to ampicillin, therefore, no statistical association could be performed between the sociodemographic data and these antibiotics.

Table 7: Association of patient demographics with antimicrobial resistance and current medications

Demographics	***p*-values of statistical association of patients' parameters against antimicrobial resistance**							**Current medication vrs Demographics**
	ATM	AK	CN	CRO	CXM	C	CIP	
Age	0.456	0.001	0.231	0.072	0.001	0.001	0.009	0.001
Current medication	0.017	0.002	0.001	0.197	0.001	0.113	0.008	-
Duration of therapy	0.001	0.028	0.001	0.023	0.001	0.001	0.041	0.001
Facility	0.701	0.005	0.915	0.001	0.001	0.185	0.033	0.038
Sex	0.003	0.251	0.002	0.017	0.001	0.003	0.550	0.057

ATM-30: Aztreonam 30 µg; AK-30: Amikacin 30 µg; CN 30: Gentamicin 30 µg; CRO-30: Ceftriaxone 30 µg; CXM-30: Cefuroxime 30 µg; C-30: Chloramphenicol 30 µg; CIP-5: Ciprofloxacin 5µg: A *p*-value < 0.05 indicates a significant association between parameters (at a 95% confidence interval and 0.05 margin of error)

Discussion:

Antibiotic resistance has emerged as a significant public health concern globally, including in Ghana (18). The prevalence of infectious diseases remains high in lower-middle-income countries like Ghana, primarily due to constrained health systems and widespread poverty, limiting access to effective antibiotics (19,20). Specifically, HIV patients are vulnerable to enteric bacterial infections and bacterial pneumonia due to their compromised immune systems (10). Frequent bacterial infections lead to increased antibiotic usage, causing resistance development through repeated microbial exposure to these antibiotics. This complicates treatment, leading to prolonged hospitalization, increased mortality, and substantial financial burdens on families and societies (10,21). This study investigated antibiotic resistance patterns of enteric bacteria in HIV-infected patients in secondary healthcare centres in a region in Ghana.

The study predominantly had female participants (84%), which aligns with global and national trends indicating a higher HIV prevalence among women (2,22). Majority of participants were recruited from Suntreso hospital, located in the Kwadaso district, which reported the third-highest HIV adult prevalence rate (6.40%) in Ghana in 2019 (23). Microbiological analysis showed *E. coli* as the predominant isolate, accounting for 80.0% of the confirmed isolates from stool samples, consistent with previous research reporting increased prevalence in rectal carriage of *E. coli* among HIV-infected individuals (24,25). This supports the initiation of empiric antibiotic therapy targeting *E. coli* or Gram-negative enterobacteria in diarrhoea illnesses in HIV patients. *Klebsiella pneumoniae*, a common intestinal commensal (26), were confirmed in 10.0% of the isolated bacteria, mirroring the findings of Najjuka (24). The remaining 10.0% consisted of *E. faecalis* (6.7%) and *E. cloacae* (3.3%), the latter being a member of the normal gastrointestinal flora and commonly associated with infections in immunocompromised or hospitalised patients (27).

The susceptibility test showed 100.0% resistance among *E. coli* isolates to amoxicillin/clavulanic acid and ampicillin, suggesting the production of β-lactamase enzymes that destroys these antibiotics (28). A similar study in South Africa, reported 50.0% of Enterobacteriaceae isolates produced extended-spectrum beta-lactamases (ESBL), with 90.0% of *E. coli* isolates exhibiting resistance to penicillins (29). Furthermore, Cotton et al., (30) reported 50.0% resistance rate to ceftriaxone, a third-generation cephalosporin, similar to the finding of this current study of 54.2% resistance rate to the third-generation cephalosporins.

However, *E. coli* in our study showed low resistance to aminoglycosides, carbapenems, chloramphenicol, and ciprofloxacin, mirroring the findings of Najjuka (25). Nevertheless, multi-drug resistance, defined as acquired resistance to at least one agent in three or more antimicrobial categories (30), was seen in 54% of the *E. coli* isolates in our study. In a study by Stanley (31), a similar trend was reported where about 57% of *E. coli* isolates were MDR. Other studies also reported a high resistance to amoxicillin/clavulanic acid, ampicillin, and 3rd and 4th generation cephalosporins in combination with tetracycline and cotrimoxazole (32,33). The resistance to cotrimoxazole may be a result of the wide use of the drug for prophylaxis in HIV care (34).

Klebsiella pneumoniae isolates showed resistance to both amoxicillin/clavulanic acid and ampicillin and were highly susceptible to ceftriaxone, cefuroxime, gentamicin, meropenem, tetracycline, chloramphenicol and ciprofloxacin. *K. pneumoniae* has been shown to have high susceptibility to aminoglycosides (35,36). The *Klebsiella* genus has inherent resistance to ampicillin due to its carriage of chromosomally located beta-lactamase gene SHV-1 (31).

The two *E. faecalis* isolates, which were both MDR, showed resistance to all the selected antibiotics except ciprofloxacin. Enterococci species are known to demonstrate intrinsic resistance to cephalosporins, amino-

glycosides, tetracycline and chloramphenicol. They are also known to have acquired genetic determinants that confer resistance to many classes of antimicrobial agents such as cephalosporins, aminoglycosides, tetracycline and chloramphenicol (37,38). *Enterobacter cloacae* isolate was resistant to aztreonam, amoxicillin-clavulanic acid, ampicillin and tetracycline. *E. cloacae* are generally more susceptible to fluoroquinolones, chloramphenicol, aminoglycosides, tetracyclines and carbapenems. On the other hand, most of them are intrinsically resistant to β-lactams owing to the production of constitutive AmpC β-lactamase (39). Resistance of *Enterobacter* to 3rd generation cephalosporins and aztreonam result from mutations in AmpD, which causes the constitutive hyperproduction of AmpC (40).

There was statically significant association ($p<0.01$) of resistance to cefuroxime, gentamicin and ciprofloxacin with the duration of therapy and the current medications of participants. This can be attributed to the fact that these medications are readily available, inexpensive drugs used to empirically treat common infections caused by a wide variety of organisms (41). The current medication of the participants was not significantly associated ($p>0.05$) with isolation of MDR pathogens. One of the potential causes of the observed high level of antibiotic resistance could be the misuse of antibiotics, both in the community and hospital settings (19).

Clinically, there are reports indicating the inappropriate prescribing practices as a cause of increased drug resistance. The wrongful use and prescription of antibiotics without microbial culture and sensitivity testing, and the unavailability of functional microbiology laboratories in most low-income countries may contribute significantly to this high prevalence in resistance (31). Another practice that leads to antibiotic resistance is patient non-compliance in completing the course of antibiotics prescribed for them (42,43), as well as the unregulated dispensing and purchase of antibiotics (mostly suboptimal doses), usually from over-the-counter facilities (31,44). The findings of our study showed high resistance to the most commonly available and affordable antibiotics but increased susceptibility to the more expensive reserve antibiotics. This may lead to increase in the cost of treatment of enteric bacterial infections in HIV patients, with increased use of effective but more expensive second line antibiotics.

A significant strength of our study was the use of MALDI-TOF and standard AST protocol, that ensured accurate identification and susceptibility of bacterial isolates. However, the study is limited by the small sample size due to the low prevalence of HIV (2%) in the region of study. This restricts the generalizability of our findings. The study was carried out in a few secondary hospitals, which might not be an accurate representation of other facilities.

Conclusion:

The enteric bacterial isolates recovered from the study population showed a predominance of *E. coli* and *K. pneumoniae*. Antimicrobial susceptibility testing showed that *E. coli* exhibited high resistance to β-lactams (amoxicillin/clavulanic acid and ampicillin) but were susceptible to ciprofloxacin and meropenem. A substantial proportion (60.0%) of the enteric isolates exhibited multidrug resistance (MDR), primarily characterised by concurrent resistance to β-lactams and cephalosporin classes, in combination with resistance to other antimicrobial agents.

These findings underscore the imperative for stringent antibiotic stewardship policies and rigorous monitoring of antimicrobial usage among HIV-positive individuals. Also, healthcare providers should emphasise patient education and counselling to combat self-medication with antibiotics and mitigate the emergence of MDR pathogens.

Contributions of authors:

DOM carried out the study. VEB and CA designed and supervised the study. DOM and YDB analysed data and drafted the original manuscript. VEB, YDB and CA edited the final manuscript. All the authors read and approved the final manuscript.

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Authors declare no conflict of interest.

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