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High level of colonization with third-generation cephalosporin-resistant *Enterobacterales* in African community settings, Ghana

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ABSTRACT

A cross-sectional survey was conducted in eight Ghanaian communities to investigate the extent of intestinal colonization with 3^{rd}-generation cephalosporin-resistant Enterobacterales. The study collected faecal samples and corresponding lifestyle data from 736 healthy residents to assess the occurrence of cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae*, with a focus on genotypes of plasmid-mediated ESBLs, AmpCs, and carbapenemases. The results showed that 371 participants (50.4%) carried 3^{rd}-generation cephalosporin-resistant *E. coli* (n=362) and K. pneumoniae (n=9). Most of these were ESBL-producing *E. coli* (n=352, 94.9%), carrying CTX-M genes (96.0%, n=338/352), mostly for CTX-M-15 (98.9%, n=334/338). Nine participants (1.2%) carried AmpC-producing *E. coli* that harboured bla_{DHA-1} or blaCMY-2 genes, and two participants (0.3%) each carried a carbapenem-resistant *E. coli* that harboured both bla_{NDM-1} and bla_{CMY-2}. Quinolone-resistant O25b: ST131 *E. coli* were recovered from six participants (0.8%) and were all CTX-M-15 ESBL-producers. Having a household toilet facility was significantly associated with a reduced risk of intestinal colonization (adjusted odds ratio, 0.71; 95% CI, 0.48-0.99; P-value=0.0095) in multivariate analysis. These findings raise serious public health concerns, and effective control of the spread of antibiotic-resistant bacteria is possible by providing better sanitary conditions for communities.

1. Introduction

The variety of β-lactamase enzymes, with their increasing substrate diversity and wide dissemination, constitute the most important antimicrobial-resistant mechanism in gram-negative bacteria [1,2]. Of these enzymes, the most clinically important are ESBLs and AmpCs that hydrolyze most penicillins and cephalosporins but not carbapenems; and lately, the carbapenemases that may inactivate carbapenems together with other beta-lactam antimicrobials. The class D carbapenemases of bla_{OXA-48} type however hydrolyze carbapenems poorly, but not the extended-spectrum cephalosporins. Often these enzymes appear in multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* that additionally are resistant to other classes of antibiotics. *E. coli* strains may be assigned to 1 of the 8 phylogroups (A, B1, B2, C, D, E, F, and the *Escherichia* cryptic clade I [3]. Strains of these groups may differ in virulence potential depending on their phylogenetic profiles [4,5].

Recently the association of ESBLs, AmpCs, and carbapenemases with epidemic clones of *Enterobacterales* have increasingly gained prominence. The most notable of these is the fluoroquinolone-resistant *E. coli* O25b-ST131 clone which is of significant public health concern because of its predominantly multidrug-resistant phenotype, and rapid dissemination as a worldwide pandemic clone. The spread of these pathogens in the community presents a huge public health concern [6,7]. Most gut colonization studies in the community have focused on ESBLs [1,2], and a few on carbapenemases [8], whilst reports of AmpCs in commensal isolates from healthy community persons are uncommon [9]. Only rarely did studies appropriately measure the occurrence of fecal isolates of cephalosporin-resistant *Enterobacterales* in an African community setting. A single study

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reported in parallel the fecal carriage of ESBL-, AmpC-, and carbapenemase-producing *E. coli* or *K. pneumoniae* in noninstitutionalized persons [10,11]. In Ghana, monitoring of antibiotic use is poor, with nascent surveillance systems and scarce reporting. In a recent commentary on antibiotic resistance in Ghana, Gyansa-Lutterodt identified a strategic need to build capacity for research into key issues related to antibiotic resistance [12]. In 2009, the ADMER program was initiated to strengthen the surveillance of antimicrobial resistance in Ghana. One of its objectives was to build research capacity and determine the prevalence, molecular characterization, and factors associated with cephalosporin-resistant *Enterobacterales* in Ghana. This is crucial for monitoring emerging trends in resistance at the local level to support clinical management.

In this study, we report the occurrence of cephalosporin-resistant *E. coli* and *K. pneumoniae*—with a particular focus on genotypes of plasmid-mediated ESBLs, AmpCs, and carbapenemases. The presence of phylogenetic clones was assessed and the emerging O25b:ST131 clone was determined. Furthermore, risk factors for intestinal colonization with these resistant bacteria were analyzed.

2. Methods

2.1. Study settings

The study was conducted among selected groups of residents in 3 groups of Ghanaian communities: (1) Korle Gonno, an urban community in the Greater Accra region of Ghana; (2) Gbese and Lanor, 2 rural communities in the Dangme West District of Greater Accra region; and (3) across 6 communities in the Tamale Metropolitan Area (i.e., Kalpohini, Taha, Bilpela, Yong, Sugashie, and Lahagu). All 8 communities were located in geographically distant regions across Ghana (Fig. 1). The choice of study sites was dependent partly on the presence of institutions able to provide personnel and expertise to help conduct field research, and partly on the receptiveness of the localities to community-based research programs.

Korle-Gonnor is an urban settlement with a land area of 250 by 850 meters located in the southern part of Ghana in the capital Accra, a few hundred meters from the nation's largest health facility, Korle-Bu Teaching Hospital. It has an estimated population of 60,000 people with a median age of 28 years, and a mixed religious people of mostly Christians, Muslims, and Traditionalists [13]. Approximately 84% of the population has attained at least a primary education. Several health facilities are available to people in this community, including the Korle-Bu Polyclinic and the Korle-Bu Teaching Hospital. Gbese and Larnor are adjacent rural communities in the Dangme West District of the Greater Accra Region. Gbese has a population size of about 1270. Lanor has approximately 414 inhabitants. One health facility, a licensed chemical shop engaged in the retail supply of over-the-counter drugs, and occasional drug peddlers are the immediate sources of medicine for both communities [14]. Tamale is located 600 km north of the Greater Accra region, near the border on the West to Togo and on the north to Burkina Faso. It has a population of over 290,000 and serves as the administrative municipality of the Northern region of Ghana. Tamale has an adult literacy rate of below 50.0% and most residents are Muslims [15]. The 6 sampled communities in Tamale were selected across the 3 sub-districts health centers in the municipality.

2.2. Study design

We conducted a community-based cross-sectional survey, from April through September 2011, to collect epidemiological data and fecal samples for microbiological investigations. Within each community, sampling involved 2 steps: (1) identifying households, selecting respondents and interviewing them; and (2) collecting fecal samples from interviewed respondents for laboratory studies. The study population comprised community residents recruited into the study based on the following criteria: elected as a respondent in a selected household; provided written consent for participation, and willing and fully capable of providing stool samples. In all 8 communities, households were selected by systematic random sampling using the Kish Method [16] which also statistically allowed for equal chances of selecting any member of the household. Structured questionnaires were used for interviews, and included data regarding demography (age, gender, number of household contacts, educational level, etc.), meals outside the home within the past 3 months, antibiotic use, hospital contacts within the past 3 months, and admissions within 1 year were collected. Other variables recorded include respondents' practices within the past 3 months (handling raw meat and the number of days handling raw meat, consumption of meat and number of days consuming meat, handling vegetables and the number of days handling vegetables, consumption of vegetables and the number of days consuming vegetables, and antibiotic use history). Respondents self-collected and submitted stool specimens for further investigations.

2.3. Microbiological investigations

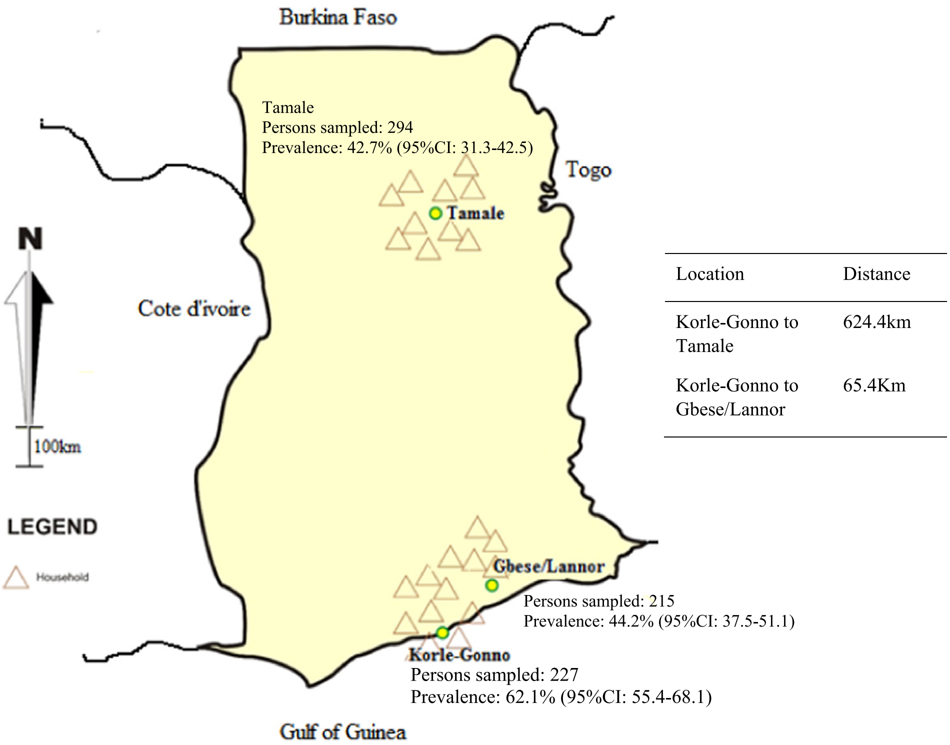
About 0.5 g of stool specimens were collected by swabs in Cary-Blair transport media (Oxoid, United Kingdom) and transported to the laboratory at the Microbiology Department of the School of Biomedical and Allied Health Sciences, Ghana, for further testing.

2.3.1. Screening of stool samples

Swabs from Cary-Blair transport media were screened for presumptive third-generation cephalosporin-resistant enterobacteria. Briefly, samples were cultured on the chromogenic Staten's Serum Institute (SSI) Enteric Media (SSI Diagnostica, Denmark) with 10 μg cefpodoxime tablets (Rosco Diagnostica, Denmark). Swab samples were also inoculated overnight in 5 mL SSI enrichment beef broth (SSI, Diagnostica, Denmark) supplemented with a 30 μg cefotaxime paper disk (MAST, UK) for additional selection of cephalosporin-resistant isolates. Enrichment broths with indications of growth after 24 hours of incubation were sub-cultured to another Enteric agar with 10 μg cefpodoxime tablets (Rosco Diagnostica, Denmark). Altogether, colonies growing around the cefpodoxime tablets within an inhibition zone of 21 mm on either SSI agar plates were defined as screen positive for third-generation cephalosporin resistance; and were subsequently identified using the biochemical test kits Minibact-E® (SSI, Diagnostica, Denmark). From these agar plates, the predominant *Enterobacterales* isolates were chosen for further study, preferably from the primary agar plates directly inoculated with the stool sample.

2.3.2. Phenotypic detection of ESBLs, AmpCs, and carbapenemases

Enterobacterales isolates positive in the screening test were examined for phenotypic expression of ESBLs, AmpCs, and carbapenemases. Detection of ESBL production was done by the combination disk method with cefotaxime (30 μg) and ceftazidime (30 μg) alone and in combination with clavulanic acid (10 μg) using NeoSensitabs (Rosco, Diagnostica, Denmark). Isolates resistant to cefpodoxime (30 μg), cefotaxime (30 μg), or ceftazidime (30 μg) according to breakpoints guidelines of the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) [17] were noted as third-generation cephalosporin-resistant isolates. A zone difference of $\geq$ 5 mm between the single and the combination disks for cefpodoxime or cefotaxime or ceftazidime was regarded as positive for ESBL production. *K. pneumoniae* ATCC 700603 was used as a positive control for ESBL production. AmpC expression was suspected in isolates with reduced susceptibility (inhibition zone $<$ 20 mm) to cefoxitin (30 μg). AmpC confirmation was performed with NeoSensitabs using



Location	Distance
Korle-Gonno to Tamale	624.4km
Korle-Gonno to Gbese/Lannor	65.4Km

Fig. 1. Prevalence of fecal carriage of *E. coli* or *K. pneumoniae* positive for AmpC, ESBL, and carbapenemases in different community settings in Ghana.

cefotaxime (30 μg) and ceftazidime (30 μg) with and without boronic acid (250 μg). A boronic acid effect of $\geq$ 5 mm in zone diameter was considered a positive result, according to the manufacturer's instructions. Reduced susceptibility to 10 μg ertapenem (inhibition zone $<$ 24 mm), 10 μg imipenem (inhibition zone $<$ 21 mm), or 10 μg meropenem (inhibition zone $<$ 21 mm) was used to screen for carbapenemase expression. Screen-positive isolates were confirmed for class A and B carbapenemases using boronic acid (600 μg) and EDTA (750 μg) effect on meropenem (10 μg) and imipenem respectively. Isolates with a boronic acid or EDTA effect of $\geq$ 5 mm in zone diameter were respectively noted as positive for class A or B carbapenemase type. Isolates with reduced susceptibility to meropenem or ertapenem or imipenem but not susceptible to temocillin (30 μg) were considered positive for class D carbapenemase. Carbapenemase-screen-positive strains were also subjected to the Modified Hodges Test (MHT) as recommended by the Clinical and Laboratory Standard Institute (CLSI) [18].

2.3.3. *Antimicrobial susceptibility testing*

E. coli and *K. pneumoniae* with ESBL, AmpC, or carbapenemase phenotype were tested for susceptibility to 17 antibiotics using the Kirby-Bauer disc diffusion method according to guidelines by EUCAST [17]. The antibiotics used were ampicillin (10 μg), mecillinam (10 μg), cefotaxime (30 μg), ceftazidime (30 μg), piperacillin/tazobactam (100/10 μg), meropenem (10 μg), imipenem (10 μg), ertapenem (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), trimethoprim (100 μg), sulphonamide (240 μg), gentamicin (10 μg), amikacin (30 μg), nalixidan (30 μg), ciprofloxacin (5 μg), and nitrofurantoin (100 μg). *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as control strains.

2.3.4. *Detection of ESBL, AmpC, and carbapenemase genes*

Isolates with, ESBL, AmpC, or carbapenemase phenotype were subjected to PCR and sequencing. See supplementary data 1 for amplification primers and conditions. All PCR protocols included an initial denaturation of 94 °C for 15 min and a final extension at 72 °C for 10 min. All AmpC phenotypes were examined for 6 families of plasmid-mediated genes including MOX (MOX-1, MOX-2, CMY-1, and CMY-8 to CMY-11), CIT (LAT-1 to LAT-4, CMY-2 to CMY-7, and BIL-1), DHA (DHA-1 and DHA-2), ACC, EBC (MIR-1T and ACT-1), and FOX (FOX-1 to FOX-5b) using the multiplex PCR assay described by Perez-Perez and Hanson [19]. A CMY-monoplex PCR, previously described by Haldorsen et al [20], was performed for all CIT AmpC positive isolates using conditions as for CIT AmpC PCR. For the detection of ESBL genes, SHV, TEM, OXA-2, OXA-10, and the CTX-M group 1, 2, and 9 primers were used. All carbapenem-resistant *Enterobacterales* were subjected to PCR assays for Class A, B, and D carbapenemases genes. A multiplex PCR assay was done to identify 5 Class A carbapenemase genes (GES, KPC, SME, INI-NMC-A). For Class B carbapenemases genes, a multiplex PCR assay was performed to differentiate between 6 metallo-beta-lactamase genes (IMP, VIM, GIM, SPM, SIM, and NDM-1). For Class D carbapenemases, carbapenem-resistant isolates were analyzed for OXA-48-like genes.

All PCR products were analyzed by horizontal gel-electrophoresis in a 2% (weight/volume) agarose gel (SeaKem®GTG®Agarose, Lonza). PCR products were purified using NucleoFast 96 PCR plates (Macherey-Nagel GmbH & Co. KG, Germany) and sent to Macrogen (Seoul, Korea) for nucleotide sequencing. Additional internal primers (Supplementary Data 1) were used for sequencing CTX-M-1, CTX-M-9, SHV, TEM, and CMY genes. DNA sequences were analyzed using Bionumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Nucleotide- and deduced protein sequences were compared with sequences in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST). TEM and SHV beta-lactamase sequences were compared to wild-type *E. coli* TEM-1 and SHV-1 sequences (GenBank accession no. AF427133.1 and AF148850 respectively) by using the database at http://www.lahey.org/studies.

2.3.5. *Determination of phylogenetic groups*

E. coli isolates with positive phenotype for ESBL, AmpC, or carbapenemase were assigned to the 4 main phylogenetic groups (A, B1, B2, and D) using methods developed by Clemont et al [21]. Three DNA markers (chuA, yjaA, and TSPE4.C2) (Supplementary Data 1) were amplified with HotStar Taq Master Mix (Fermentas, Germany) at an initial denaturation of 94 °C for 4 minutes and a final extension step at 72 °C for 7 minutes. The following were used as control strains: *E. coli* MG16555, *E. coli* J96, *E. coli* EDL 933, and *E. coli* CFT 073.

2.3.6. Detection of O25b-ST131 clone

An allele-specific PCR assay, developed by Clermont et al [22] and based on nucleotide sequence polymorphisms in the *pab*B and *trp*A genes specific to the B2 subgroup I/O25b *E. coli* strains was performed to detect O25b-ST131 *E. coli* clones. Briefly, a 347 bp fragment of *pab*B gene-specific to O25b-ST131 and 427bp fragment of the *trpA E. coli* gene used by Institut Pasteur MLST *E. coli* scheme were amplified with Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) using an initial denaturation step of 94 °C for 15 minutes and a final extension at 72 °C for 10 minutes.

2.4. Statistics

Data from interviews and laboratory investigations were entered into a Microsoft ACCESS database and imported into Statistical Package for Social Sciences (SPSS, Version 20.0) for editing and statistical analyses. Isolates were categorized as multidrug resistant bacteria (MDRB) if they were resistant to at least 1 agent in 3 or more antimicrobial categories (aminoglycosides, fluoroquinolones, penicillins, cephalosporins, antipseudomonal penicillins/β-lactamase inhibitor, folate-pathway inhibitors, tetracycline, phenicols). Continuous data were compared using a *t*-test where appropriate. Point estimates of statistical significance were 2-tailed at *P*-values <0.05. Categorical data were compared across study parameters using $\chi 2$ or Fisher's exact tests. Correlations were assessed with the Pearson coefficient (r) or Spearman's rho (r_s) and their coefficient of determination (r^2 or $r_s{}^2$) where appropriate. Line graphs depicting trends were constructed with Locally Weighted Scatterplot Smoothing (LOESS) Tricube kernel smooth fit line with 50% of points to fit. The association between respondents infected with, ESBL-, AmpC-, or carbapenemase-producing *E. coli* and *K. pneumoniae* and their independent predictor variables were quantified by odds ratio (OR) with a 95% confidence interval (CI). From univariate analyses, variables with a *P*-value <0.05 were analyzed in multivariate logistic regression models to identify independent risk factors. The predictive accuracy of the models was evaluated by Hosmer and Lemeshow's goodness-of-fit test with a *P*-value >0.05 suggesting that the model predicts accurately on average. The area under the ROC (Receiver Operating Characteristic) Curve > 0.7 was used to analyze the discriminatory capability of the models.

2.5. Ethical considerations

Approval (No. F1123ha468) for research was obtained from the Ethical Review and Protocol Committee of the University of Ghana Medical School, Korle-Bu. Before the commencement of work, the research team conducted meetings with community members of the selected study area to explain the objectives of our study including benefits, potential risks, and discomforts. Respondents provided written consent before enrolment into this study. Informed written consent for children was sought from parents or legal guardians. In addition, children were requested to give assent to their involvement; with the right to refuse participation or withdraw at any time during the study. Stool samples and accompanying data were de-identified and allotted arbitrary numbers to ensure anonymity.

3. Results

Overall, 1020 noninstitutionalized community residents (1 per household) were contacted. None of the participants lived in the same household as another. In total, 736 community residents provided stool specimens (response rate, 74.5%)—227 from Korle-Gonno, 215 from Gbesse and Lannor, and 294 from Tamale. It was recorded that 43% (*n* = 237/552) of the households were without toilet facilities, and 61% of participants (369/605) had no fitted pipe-borne water in their homes. The mean age of participants was 35.4 ± 26.1 years, the female-to-male ratio was 2.35, and the average number of persons per household was 6.31 ± 3.42. About 43% (*n* = 67/156) of the participants used antibiotics without a prescription when in need of treatment. Third-generation oral cephalosporins accounted for 43.5% of the antibiotics used. Data regarding demographics, including housing conditions and the educational status of participants are shown in Table 1. There was no significant age difference between participants from the 3 study areas. Participants from Korle-Gonno had significantly better education and employment status and had fewer persons in a household.

3.1. Cephalosporin-resistant isolates with ESBL, AmpC, or carbapenemase phenotype

In total, 382 (51.9%) out of 736 participants were colonized with third-generation cephalosporin-resistant *Enterobacterales*. Three hundred and sixty-two (94.7%) had *E. coli*, 9 (2.4%) had *K. pneumoniae*, 4 (1.0%) had *Citrobacter freundii*, 3 (0.8%) had *Serratia marcescens*, 2 (0.5%) had *Enterobacter cloacae*, and 1 (0.25%) each had *Klebsiella oxytoca* and *Morganella morgani*. The 11 non-*E. coli* and non-*K. pneumoniae* were excluded from the study (Fig. 2). The remaining 371 cephalosporin-resistant *E. coli* (*n* = 362) and *K. pneumoniae* (*n* = 9) were included in subsequent investigations for expression of AmpC, ESBL, and carbapenemase. The breakdown of numbers by study site, species, and type of β-lactamases conferring third-generation cephalosporin resistance are shown in Table 2. Altogether, 360 of the 371 cephalosporin-resistant *E. coli* and *K. pneumoniae* were phenotypically positive for AmpC, ESBL, or carbapenemases. Of these, 351 were ESBL-producers only (349 *E. coli*; 2 *K. pneumoniae*), 2 expressed only AmpC phenotype (all *E. coli*), 5 had ESBL plus AmpC phenotype (all *E. coli*), and 2 *E. coli* isolates resistant to carbapenem antibiotics expressed AmpC plus Class B carbapenemase phenotypes (Table 2 and Fig. 2). None of the isolates was phenotypically positive for Class A or OXA-48-type carbapenemases. The 11 cephalosporin-resistant *E. coli* (*n* = 4) and *K. pneumoniae* (*n* = 7) were negative for ESBL, AmpC, or carbapenem-resistant phenotype and were excluded from subsequent PCR and sequencing. Fig. 1 shows the prevalence of fecal carriage across the 3 geographically distant study regions. Carriage occurred more frequently in the city than in other areas. Our data represent a colonization prevalence of 48.3% for ESBL [*E. coli* (47.6%, *n* = 351/736); *K. pneumoniae* (0.6%, *n* = 5/736)], 1.2% (*n* = 9/736) for AmpC, and 0.3% (*n* = 2/736) for carbapenemases.

3.2. Antibiogram and multidrug-resistant isolates

The antibiogram of the above 360 isolates is shown in Table 3. There were no differences among the 3 study regions. Seventy-seven per cent (*n* = 277/360) of the isolates were multidrug-resistant bacteria. The majority (99.3%) of the MDRB were susceptible to the carbapenems nitrofurantoin (91%), mecillinam (82%), and gentamicin (74%).

3.3. Genotypic characterization of ESBL, AmpC, and carbapenemase genes

A corresponding gene was identified in all 360 phenotypically confirmed ESBL, AmpC, or carbapenemase isolates. Overall, 99.4%

(*n* = 354/356) of isolates with ESBL phenotype carried $\textit{bla}_{\text{CTX-M}}$ gene (Korle-Gonno, 98.6%, *n* = 138/140; Gbese/Lannor, 100.0%, *n* = 92/92; Tamale, 100% *n* = 124/124) (Table 4). All CTX-M-producers were *E. coli*, and nearly all belonged to the CTX-M-1 group with $\textit{bla}_{\text{CTX-M-15}}$ as the predominant gene found in 95.7% (*n* = 134/140), 100% (*n* = 92/92), and 100% (*n* = 124/124) of the ESBL isolates from Korle-Gonno, Gbese/Lannor, and Tamale respectively. The 2 *K. pneumoniae* isolates included in the analysis all harbored $\textit{bla}_{\text{SHV-31}}$. All 176 $\textit{bla}_{\text{TEM}}$-positive isolates were also $\textit{bla}_{\text{CTX-M}}$-positive. Of these, 175 isolates carried the non-ESBL TEM-1 gene whilst one isolate carried the ESBL TEM-190 gene. We identified $\textit{bla}_{\text{DHA-1}}$ (*n* = 1) and $\textit{bla}_{\text{CMY-42}}$ (*n* = 1) in the 2 *E. coli* with AmpC-only phenotype. Of the 5 isolates with AmpC plus ESBL phenotype, 2 carried $\textit{bla}_{\text{CMY-42}}$ AmpC with $\textit{bla}_{\text{CTX-M-15}}$ ESBL genes whilst 3 harbored $\textit{bla}_{\text{DHA-1}}$ AmpC genes plus $\textit{bla}_{\text{CTX-M-15}}$ ESBL genes. The 2 isolates with carbapenemase and AmpC activity both carried $\textit{bla}_{\text{NDM-1}}$ and $\textit{bla}_{\text{CMY-42}}$. None of the isolates harbored Class A or OXA-48-like carbapenemases.

3.4. Variations in ESBL, AmpC, and carbapenemases genes across study populations

The 3 study regions showed some differences in the distribution of ESBLs, AmpCs, and carbapenemase genotypes. Isolates from Korle-Gonno showed greater diversity in ESBL genotypes—the 140 *E. coli* and *K. pneumoniae* ESBLs belonged to 7 $\textit{bla}_{\text{ESBL}}$-type variants. In contrast, only the CTX-M-15 gene was found in Gbese/Lannor and Tamale. Note also that isolates with the AmpC ($\textit{bla}_{\text{DHA-1}}$, $\textit{bla}_{\text{CMY-42}}$) and carbapenemase genes ($\textit{bla}_{\text{NDM-1}}$) were not identified in Tamale, despite being reported in Korle-Gono and Gbese/Lannor. Overall, the prevalence of CTX-M-type ESBL-producers was significantly higher in Korle-Gonno and Tamale than in Gbese/Lannor (*P-value* = 0.003). Similar proportions of AmpC genotypes were found in Korle-Gonnor (3.5%; *n* = 5/141) and Gbese/Lannor (3.1%, *n* = 3/95), but none in Tamale

3.5. Phylogenetic groups for ESBL, AmpC, or carbapenemase-producing E. coli

The phylogenetic group was determined for all 358 phenotypic test-positive *E. coli* included in the study (Fig. 2). The phylogenetic group distribution did not differ between study regions or by ESBL type (Table 4). The AmpC producers were predominantly of phylogenetic group A (57%, *n* = 5/7). In all study regions, phylogroup A (61%-75%) was most common among the ESBL-producers followed by group B1 (34%-43%). Few (1-7%) phylogroup B2 isolates were found. The 2 carbapenemases-producing isolates that harbored both $\textit{bla}_{\text{NDM-1}}$ and $\textit{bla}_{\text{CMY-42}}$ genes belonged to phylogroups A and B1, respectively.

3.6. Quinolone resistant strains

Two hundred and twenty-one *E. coli* isolates, all with ESBL phenotype, were resistant to nalidixic acid (Nal-R). 67% (*n* = 147) of these were resistant to ciprofloxacin. Six Nal-R isolates (4 of which were ciprofloxacin-resistant) belonged to group B2 and were also confirmed to be O25b.ST131 strains.

3.7. Risk factors for community fecal carriage

Using univariate analysis, we found the following factors to be associated with the risk of fecal carriage of *E.coli* and *K.pneumoniae* positive for ESBL, AmpC or carbapenemase (Table 5): age beyond 65 years, crop or livestock farming as an occupation, no toilet facility in the household, number of contact persons in the household, history of hospital visits in past 1 year, use of antibiotics within the past 3 months, and cephalosporin use within the past 3 months.

The final multivariate logistic regression model identified 3 independent variables (Table 5) associated with fecal carriage in the community: (1) the availability of toilet facilities in households (AOR, 0.71; 95%CI, 0.48-0.99; *P*-value = 0.0095); (2) antibiotic treatment in past 3 months (AOR, 3.61; 95%CI, 2.23-5.31; *P-value* = 0.0001); and (3) person's age > 65 years (AOR, 3.76; 95%CI, 2.64-5.71; *P-value* = 0.0001). Fig. 3 displays the association between age and the incidence of fecal colonization by the 360 phenotypic tests positive Cephaosorin Resistant isolates. Over the life span, the occurrence of fecal colonization did not correlate with age (r = 0.113; r^2 = 2.3%; *P-value* = 0.069); but among those aged ≥ 65 years, an apparent linear trend of increasing incidence of fecal carriage was observed with age. Pearson correlation coefficient showed this relationship to be significant (r = 0.314; r^2 = 9.8%; *P-value* = 0.021). The rate of increase of incidence appears to be more pronounced for respondents beyond 65 years.

Table 1
Baseline characteristics of 736 respondents who provided stool specimen for study.

Characteristics (*n*, total respondents)	Number of respondents per study site[c]		
	Korle-Gonno (*n* = 227)	Gbesse/Lannor (*n* = 215)	Tamale (*n* = 294)
Male gender (*n* = 211)	76 (33.5%)[a]	87 (40.5%)[a]	48 (18.1%)[b]
Mean age ± SD	34 ± 37.3[a] (range, 0.5-100 years)	35.0 ± 42.3[a] (range, 7-115 years)	36 ± 23[a] (range, 2-80 years)
No formal education (*n* = 89)	15 (6.8%)[a]	29 (13.6%)[b]	45 (15.3%)[b]
Unemployed (*n* = 263)	85 (47.7%)[a]	91 (56.5%)[a]	87 (80.5%)[b]
Crop farming as occupation (*n* = 146)	56 (27.2%)[a]	49 (24.0%)[a]	41 (15.2%)[b]
Livestock farming as occupation (*n* = 107)	35 (16.9)[a]	42 (20.6%)[a]	30 (10.2%)[b]
Toilet facility at home (*n* = 311)	118 (56.4%)[a]	100 (50.7%)[a]	93 (63.7%)[b]
Has tap water at home (*n* = 297)	105 (48.8%)[a]	94 (49.2%)[a]	98 (49.3%)[a]
Visited a hospital within last year (*n* = 463)	216 (83.9%)[a]	194 (88.0%)[a]	53 (30.1%)[b]
Admitted to hospital within last year (*n* = 44)	12 (9.2%)[a]	18 (10.0%)[a]	14 (9.1%)[a]
Household diarrhoae in 3 months (*n* = 81)	20 (14.8%)[a]	25 (13.5%)[a]	36 (16.3%)[a]
Used antibiotics within last 3 months (*n* = 156)	80 (53.7%)[a]	66 (55.5%)[a]	10 (12.4%)[b]
Used antibiotics without prescription in last 3 months (*n* = 67)	27 (48.2%)[a]	34 (43.3%)[a]	6 (34.4%)[a]
Mean number of persons in household ± SD	4.4 ± 3.1[a] (range, 3-9)	6.3 ± 4.2[b] (range, 2-11)	7.1 ± 5.1[b] (range, 4-15)

SD = standard deviation.

[a,b] Differing superscripts within a row show significant difference between categories, where b > a at $P < 0.05$.

[c] %, percentage (number of respondents characterized by an option in a questionnaire variable divided by the total respondents for that variable). The number of respondents for each variable differ (i.e., missing values were excluded from analysis.)

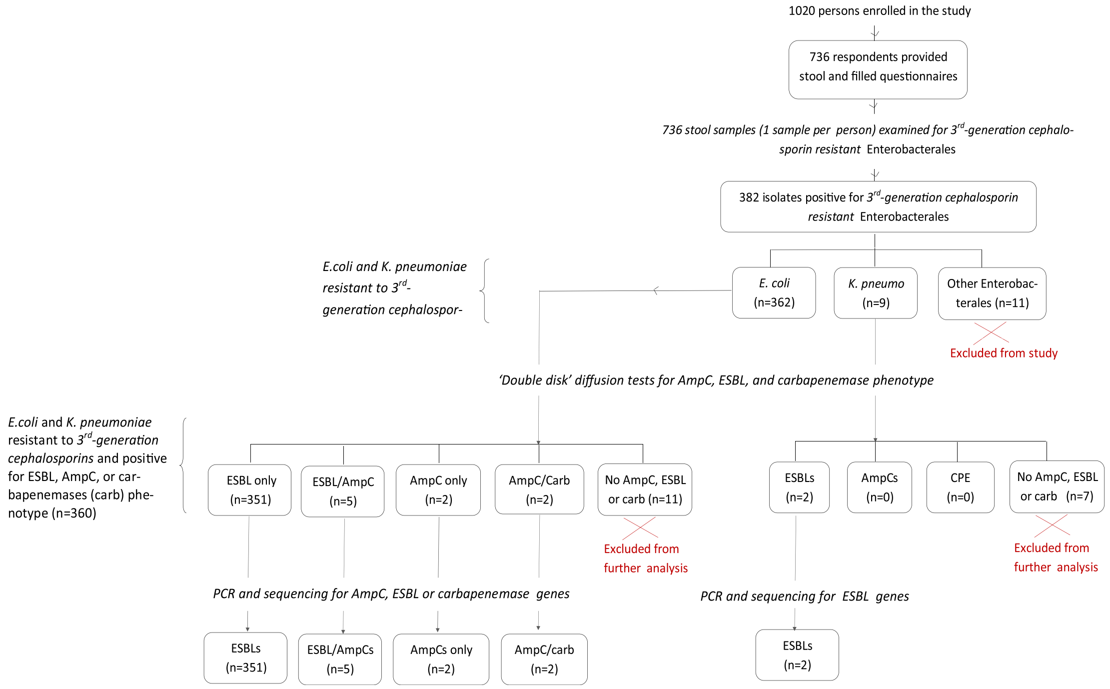


Fig. 2. Summary for sampling, laboratory procedures, and outcomes.

4. Discussion

The main finding of this study is a high rate (50.4%) of intestinal colonization with cephalosporin-resistant *Enterobacterales* across communities in Ghana. There was a predominance of intestinal colonization with ESBL-positive *E. coli*, most of which carried *bla*$_{\text{CTX-M-15}}$ genes. AmpC-producing strains were relatively uncommon, and they carried the *bla*$_{\text{CMY-2}}$ and *bla*$_{\text{DHA-1}}$ genes. A few participants were colonized with carbapenem-resistant isolates, all of which harbored *bla*$_{\text{NDM-1}}$ metallo-beta-lactamase genes. In multivariate analysis, having a household toilet facility was protective against intestinal colonization. Antibiotic use and age> 65 years were identified as predisposing factors.

The findings highlight communities as a significant reservoir of ESBLs in Ghana. Two observations merit attention. First, the carriage prevalence of ESBL-producers (48.3%) observed in this study is higher than that reported in studies spanning across sub-Saharan Africa, most conducted in hospital settings [10,11,23-28]. In northern Africa, the estimated prevalence of ESBL carriage was 42.8% among 77 patients admitted to medical units of a University hospital in Morocco [29]. It was even higher (63.3%) among 632 asymptomatic individuals attending a checkup clinic in Cairo, Egypt [30] but still lower than the estimate reported here for Kalpohini community in the Tamale Metropolitan Area. The carriage rate was higher in the city than in other areas. This could be due to the nearness and easy access of this study site to several hospitals, particularly the KBTH, which is Ghana's premiere and largest tertiary-level hospital [12]. Hospitals are a repository of resistant bacteria and serve as a conduit of antimicrobial pathogens to the community [6,7].

The strength of our study is that all participants were noninstitutionalized community residents randomly and systematically enrolled from various locations across the country. The to-date reported studies include persons either admitted or attending hospitals. Using the comparable methodology, our estimates are much higher than the carriage prevalence reported across Europe (<10%) [31–38] and the United States (<5%) [39,35] but similar to data from the Western Pacific

Table 2
Fecal colonization with third-generation cephalosporin resistant *E. coli* and *K. pneumoniae* and types of resistant mechanisms.

Study site (number of respondents)	[a]Respondents colonized by third-generation cephalosporin resistant isolates	Type of resistant mechanism[b]								
		ESBL		ESBL +AmpC		AmpC		AmpC + carbapenemase		[a]Total
		Phenotypic detection	PCR and sequencing	Phenotypic detection	PCR and sequencing	Phenotypic detection	PCR and sequencing	Phenotypic detection	PCR and sequencing	
Korle-Gonno (*n* = 227)	144 (63.4)	136 (59.9)	136	4 (1.7)	4	0	0	1 (0.4)	1	141 (62.1%)
Gbese and Lannor (*n* = 215)	99 (46.1)	91 (46.1)	91	1 (0.5)	1	2 (1)	2	1 (0.5)	1	95 (44.2%)
Tamale (*n* = 294)	128 (43.5)	124 (42.2)	124	0	-	0	-	0	-	124 (42.7%)
-Kalpohini (*n* = 50)	35 (70.0)	35 (70.0)	35	0	-	0	-	0	-	35 (70.0%)
-Taha (*n* = 50)	22 (44.0)	22 (44.0)	22	0	-	0	-	0	-	22 (44.0%)
-Bilpela (*n* = 50)	22 (44.0)	20 (40.0)	20	0	-	0	-	0	-	22 (44.0%)
-Yong (*n* = 50)	19 (38.0)	17 (34.0)	17	0	-	0	-	0	-	19 (38.0%)
-Sugashie (*n* = 50)	15 (30.0)	15 (30.0)	15	0	-	0	-	0	-	15 (30.0%)
-Lahagu (*n* = 44)	15 (30.0)	15 (30.0)	15	0	-	0	-	0	-	15 (34.1%)
Total (736)	371 (50.4%)	351 (47.6%)		5 (0.6%)		2 (0.3%)		2 (0.3%)		360 (48.9%)

[a] 11 third generation cephalosporin resistant *E. coli* (*n* = 4) and *K. pneumoniae* (*n* = 7) were negative for ESBL, AmpC or carbapenemase phenotype.

[b] All particaipants carried *E. coli* except 2 persons from Korle-Gonno who each were colonized with a *K. pneumoniae* isolate. The 2 *K. pneumoniae* isolates were ESBL-positive (with no AmpC and carbapenem resistance) by phenotypic test, PCR, and sequencing.

Table 3
Antibiogram of AmpC-, ESBL-, or carbapenemase-positive *E. coli* and *K. pneumoniae* across study sites.

Antimicrobials	Antibiotic resistance (%)				[c]Susceptibilty of MDRB [$n = 277$ (%)]
	Study sites			Total ($n = 360$)	
	Korle-Gonno ($n = 141$)	Gbese/ Lannor ($n = 95$)	Tamale($n = 124$)		
Ampicillin (10 μg)	141 (100)	95 (100)	-	236 (100)	0
Mecillinam (10 μg)	37 (26) [b]	6 (6)[a]	7 (5.6)[a]	50 (14)	228 (82)
Cefotaxime (30 μg)	141(100)	95 (100)	124 (100)	360 (100)	0
Ceftazidime (30 μg)	141 (100)	93 (98)	113 (91.1)	347 (96)	13 (5)
Aztreonam (30 μg)	138 (98)	93 (98)	-	231 (64)	238 (86)
Piperacillin (30 μg) /Tazobactam(6 μg)	31 (22)	24 (25)	41 (33.1)	95 (26)	39 (14)
Meropenem (10 μg)	1 (0.07)	1 (1.1)	0	2 (0.6)	275 (99)
Ertapenem (10 μg)	1 (0.07)	1(1.1)	0	2 (0.6)	275 (99)
Imipenem (10 μg)	1 (0.07)	1 (1.1)	0	2 (0.6)	275 (99)
Nalixidan (30 μg)	90 (64)	55 (58)	76 (61.3)	221 (61)	44 (16)
Ciprofloxacin (5 μg)	58 (41)	36 (38)	53 (42.5)	147 (41)	142 (51)
Trimethoprim (100 μg)	126 (90)	89 (94)	110 (88.7)	325 (90)	0
Sulphonamides (240 μg)	128 (91)	90 (95)	112 (90.3)	330 (92)	0
Tetracycline (30 μg)	114 (81)	77 (80)	106 (85,4)	297 (83)	249 (10)
Chloramphenicol (30 μg)	37 (26)	26 (27)	37 (29.8)	100 (28)	178 (64)
Gentamicin (10 μg)	31 (22)	18 (19)	14 (11.3)	63 (18)	213 (74)
Nitrofurantoin (100 μg)	13 (9)[b]	11 (12)[a]	0[a]	24 (7)	253 (91)

[a,b] Numbers with differing superscripts within a row are statistically different with b > a at $P < 0.05$.

[c] MDR = multidrug resistant AmpC, ESBLs or carbapenemase-producing isolates

[40–42] and Southeast Asia regions [43,44] showing very high carriage rates that reach up to 69.3%. ESBLs have long existed in Ghana [45] and the lack of awareness and laboratory detection may compound the problem. A high carriage rate of ESBLs increases the risk of infection with multi-resistant bacteria and has significant public health implications for international travel. Travelers returning home from areas associated with high ESBL fecal carriage are reportedly at increased risk of ESBL-positive infections [37-39,46,47]. Second, in studies from Ghana [8,26,48,49] and across Africa [10,11] where ESBL genotypes have been analyzed, CTX-M alleles, predominantly CTX-M-15, accounted for the significant majority (>60%) of ESBLs, highlighting the importance of these genes in the ongoing global dissemination of multi-drug resistant bacteria. The spread of ST131 *E.coli* clones has been widely reported in association with blaCTX-M-15 genes and parallels the global dissemination of ESBLs [50,51]. In our study, only 6 *E.coli* strains [comprising 66.7% (n = 6/7) of total B2 isolates], belonged to ST131 thus providing no evidence that ST131 *E. coli* is the main contributor to the spread of ESBLs in Ghana.

There is a dearth of community-based studies examining intestinal colonization with AmpCs and carbapenemases, limiting the scope for comparing our results with the literature. Although KPC and OXA-48-type carbapenemase genes were not identified, the carriage of NDM-1 *E.coli* among unrelated residents with no history of hospitalization or travel outside Ghana is a concern because this could very quickly start the dissemination of clones for which effective antibiotics may not be available in Ghana. The implications of NDM-1 gene

Table 4
Gene composition in *E. coli* ($n = 358$) and *K. pneumoniae* ($n = 2$) positive for AmpC, ESBL, and carbapenemases phenotype.

Gene types	*E.coli* phylogenetic groups														
	Korle-Gonno					Gbese and Lannor					Tamale				
	[a]Num	A	B1	B2	D	Num	A	B1	B2	D	Num	A	B1	B2	D
ESBLs only															
$bla_{\text{CTX-M-101}}$ ($n = 1$)	1	1	0	0	0	0					0				
$bla_{\text{CTX-M-15}}$ ($n = 328$)	130[c]	69	42	6	13	91[c]	58	19	2	12	107[c]	61	30	1	15
$bla_{\text{CTX-M-15/TEM-1}}$ ($n = 15$)	0					0					15	5	6	0	4
$bla_{\text{CTX-M-15/SHV-1}}$ ($n = 1$)	0					0					1	0	1	0	0
$bla_{\text{CTX-M-27}}$ ($n = 1$)	1	1	0	0	0	0					0				
$bla_{\text{CTX-M-9}}$ ($n = 1$)	1	1	0	0	0	0					0				
$bla_{\text{CTX-M-15/TEM-190}}$ ($n = 1$)	0					0					1	1	0	0	0
$bla_{\text{CTX-M-14}}$ ($n = 1$)	1	0	0	1	0	0					0				
[b]$bla_{\text{SHV-31}}$ ($n = 2$)	2	ND	ND	ND	ND	0					0				
ESBL plus AmpC															
$bla_{\text{CTX-M-15}}$/$bla_{\text{CMY-2}}$ ($n = 2$)	2	1	0	0	1	0					0				
$bla_{\text{CTX-M-15}}$/$bla_{\text{ DHA-1}}$ ($n = 3$)	2	1	1	0	0	1	1	0	0	0	0				
AmpCs only															
$bl_{a\text{DHA-1}}$ ($n = 1$)	0					1	1	0	0	0	0				
$bla_{\text{CMY-2}}$ ($n = 1$)	0					1	1	0	0	0	0				
Carbapenemases plus AmpCs															
$bla_{\text{NDM-1}}$/ $bla_{\text{CMY-2}}$ ($n = 2$)	1	1	0	0	0	1	0	1	0	0	0				
Total ($n = 360$)	141	75	43	7	14	95	61	20	2	12	124	67	37	1	19

ND = not done

[a] Num, number of isolates per community

[b] Gene identified in 2 *K. pneumoniae* isolates

[c] Numbers with the same superscripts within a row are significantly different at $P < 0.05$.

Table 5
Univariate and multivariate analysis of factors associated with fecal colonization with AmpC-, ESBL-, or carbapenemases-positive *E. coli* and *K. pneumoniae*.

Variables	Univariate comparisons					AOR (95%CI)	*P*-value
		Fecal carriage					
	[a]Overall no. of respondents ($n = 736$)	Yes ($n = 360$)	No ($n = 376$)	Unadjusted odds ratio (95%CI)	*P*-value		
Gender	708	347	361				
Male	211	107	104	1.10 (0.79-1.52)	0.554	-	-
Female	497	240	257	0.91 (0.66-1.25)			
Age (Mean ± SD)	703	36.8 ± 23.6	34.4 ± 22.1	1.3 (0.9-5.8)	0.164	-	-
Age group (years)	703	342	361				
0-15	228	113	115	1.01 (0.77-1.47)	0.74	-	-
15-65	319	154	165	0.97 (0.72-1.31)	0.86	-	-
>65	156	113	43	3.64 (2.47-5.39)	0.0001	3.76 (2.64-5.71)	0.0001
Farming as occupation	679	336	343				
Yes	253	139	114	1.41 (1.04-1.93)	0.028	1.21 (0.91-1.69)	0.096
No	426	197	229				
Crop farming as occupation	679	336	343				
Yes	146	82	64	1.41 (0.97-2.03)	0.068	-	-
No	533	254	279				
Livestock farming as occupation	679	336	343				
Yes	107	57	50	1.19 (0.79-1.81)	0.392	-	-
No	572	279	293				
Current smoking	572	293	279				
Yes	81	38	43	0.82 (0.51-1.31)	0.402	-	-
No	491	255	236				
Alcohol use	572	293	279				
Yes	461	242	219	1.3 (0.86-1.97)	0.214	-	-
No	111	51	60				
Underlying disease	333	168	165				
Diabeties	114	65	49	1.40 (0.88-2.21)	0.146	-	-
Cardiovascular	97	40	57	0.84 (0.52-1.35)	0.475	-	-
Gastrointestinal	81	43	38	1.1 (0.66-1.79)	0.74	-	-
Infections	41	20	21	0.88 (0.45-1.69)	0.71	-	-
Education level	718	357	361				
No formal education	89	40	49	0.83 (0.53-1.28)	0.396	-	-
Basic to high school	406	194	212	0.84 (0.62-1.1)	0.236	-	-
Tertiary	223	123	100	1.37 (0.99-1.88)	0.051	-	-
Unemployed	447	307	140				
Yes	263	184	79	1.15 (0.78-1.73)	0.483	-	-
No	184	123	61				
Toilet facility in household	552	201	351				
Yes	311	99	212	0.63 (0.44-0.90)	0.011	0.71 (0.48-0.99)	0.0095
No	241	102	139				
Flush toilet at home	532	193	339				
Yes	193	61	132	0.72 (0.49-1.04)	0.08	-	-
No	339	132	207				
Tap water at home	605	221	384				
Yes	297	103	194	0.85 (0.60-1.20)	0.362	-	-
No	308	118	190				
Number of persons in household	736	7.2 ± 3.5	3.1 ± 1.7	4.10 (3.70-4.5)	0.0001	2.02 (1.46-2.51)	0.062
Household diarrhoae in 3 months	521	282	239	0.71 (0.44-1.14)	0.156		
Yes	81	38	43				
No	440	244	196				
Hospital visits in last year	657						
Yes	463	258	205	1.53 (1.09-2.14)	0.0127	1.34 (0.95-1.87)	0.071
No	184	83	101				
Admitted to hospital in last year	463	271	192				
Yes	44	32	12	1.58 (0.82-3.07)	0.171	-	-
No	419	239	180				
Handled raw vegetables within past 3 months	451	222	229				
Yes	227	111	116	0.97 (0.67-1.41)	0.882	-	-
No	224	111	113				
Number of days handling raw vegetables		14.7 ± 7.3	16.2 ± 8.1	0.82 (0.52-3.25)	0.145	-	-
Handled raw meat within past 3 months	431	281	150				
Yes	198	101	97	0.69 (0.44-1.1)	0.101	-	-
No	233	180	53				
Number of days handling raw meat		15.4 ± 7.1	13.7 ± 8.1	1.18 (0.43-3.83)	0.118	-	-
Used antibiotics in last 3 months	339	168	171				
Yes	156	102	54	3.35 (2.14-5.23)	0.0001	3.61 (2.23-5.31)	0.0001
No	183	66	117				
[b]Type of antibiotics used in last 3 months	339	168	171				
Cephalosporins	68	39	19	2.42 (1.33-4.39)	0.003	1.93 (1.11-3.33)	0.059
Macrolides	7	4	3	1.36 (0.3-6.19)	0.721	-	-
Penicillins	26	11	15	0.73 (0.32-1.63)	0.442	-	-

(*continued*)

Table 5 (*Continued*)

Variables	Univariate comparisons					AOR (95%CI)	*P*-value
		Fecal carriage					
	[a]Overall no. of respondents (*n* = 736)	Yes (*n* = 360)	No (*n* = 376)	Unadjusted odds ratio (95%CI)	*P*-value		
Quinolones	28	13	15	0.87 (0.40-1.89)	0.729	-	-
TMP-SMX	8	3	5	0.606 (0.14-2.56)	0.723	-	-
Nitrofurantoin	5	2	3	2.59 (0.49-13.54)	0.280	-	-
Aminoglycosides	16	8	8	0.97 (0.35-2.64)	1		
Used antibiotics without prescription	156	102	54				
Yes	67	54	13	1.84 (0.93-3.67)	0.078	-	-
No	89	48	41				

SD = standard deviation; AOR = adjusted odds ratio; TM-SMX = trimethoprim-suphurmethoxazole; CI = confidence interval; cephalosporin resistant *Enterobacterales*$^{+}$, cephalosporin resistant *E.coli* and *Klebsiella pneumoniae* positive for AmpC, ESBL or carbapenemases

[a] Number of respondents for each variable differ.

[b] Number of respondents overlap across categories due to multiple responses.

transmission are thoroughly discussed by many workers [52–56]. This is, to our knowledge, the largest study of fecal carriage of ESBL-, AmpC, and carbapenemases-producing *Enterobacterales* in Africa. Similar studies have involved only ESBLs and only 1 of them investigated some risk factors [25]; there are not many from other parts of the world. Our study reports that antibiotic use—especially cephalosporins, which do not come out in multivariate analysis but are significant in univariate comparisons—increases the risk of being colonized by isolates that harbor ESBLs, AmpCs, or carbapenemases. This observation is consistent with several other case-control studies for ESBL colonization [38,57-59]. Age was also determined to be a predisposing factor. The carriage prevalence significantly increased among adults > 65 years of age (Fig. 3). In this group of persons, empirical antimicrobial usage is likely to be higher due to responsive medical attention. There is an increased risk for elderly patients, who are more prone to recurrent infections including urinary tract infections. Interestingly having a household toilet facility reduced the risk of intestinal colonization by about 30%. Public places of convenience may serve as a repository for resistant bacteria, and influence person-to-person dissemination fecal-orally [60].

Organisms producing ESBL, AmpC, or carbapenemase merit close monitoring with enhanced surveillance efforts that can track and tailor interventions targeted at stopping their dissemination. However, the absence of routine surveillance and laboratory detection of these pathogens in many clinical laboratories in Ghana compounds the problem. Although the detection of ESBLs, AmpCs, and carbapenemases remains difficult in high-income countries, the challenge is formidable in low-income countries where microbiological services are accessible only in a few clinical laboratories. Whereas many laboratories may not be fully aware of the importance of ESBL-, AmpC,

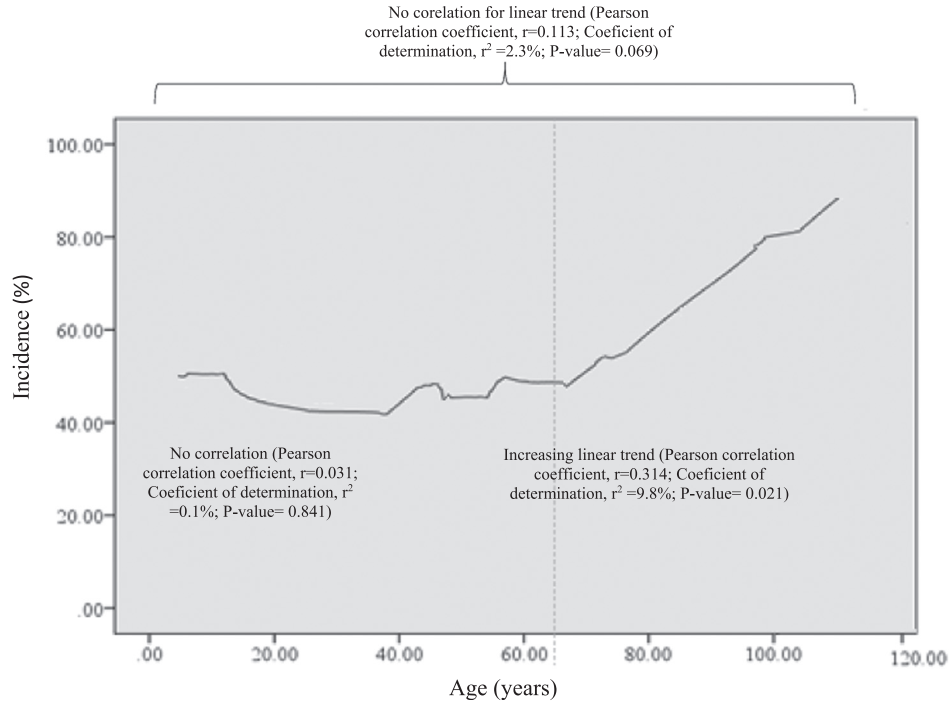


Fig. 3. Age-specific incidence of intestinal colonization bycephalosporin resistant *Enterobacterales* with positive phenotype for ESBL, AmpC, or carbapenemase. Generated with Locally Weighted Scatterplot Smoothing (LOESS) Tricube kernel smooth fit line with 60% of points to fit.cephalosporin resistant *Enterobacterales*, cephalosporin resistant *E. coli* and *K. pneumoniae*.

and carbapenemase-producing enterobacteria, others may lack the ability to correctly identify and report these organisms. Health professionals especially clinicians must be made aware of the local prevalence and the public health implications of resistant bacteria to aid rational antibiotic drug use.

Interventions including infection control measures and proper stewardship programs that restrict antibiotic use in both the community and hospitals are important in limiting the spread of these pathogens. The present work highlights the fact that judicious control of the spread of intestinal carriage of ESBL, AmpC-, and carbapenemases-producing bacteria cannot be done effectively without proper regard for better housing and sanitary conditions.

Our findings should be interpreted considering certain technical limitations. Where more than one colony type per *E. coli or K. pneumoniae* was recovered as phenotypically positive for ESBLs, only the dominating colony type was used for analysis. ESBLs, AmpCs, and carbapenemases are prevalent among various members of the *Enterobacterales* and not just *E. coli* and *K. pneumoniae* [56,61,62]. The enrichment protocol was performed with 6 mg/L of cefotaxime, which is rather high. This may lead to the underestimation of third-generation cephalosporin-resistant enterobacteria, especially those with genes conferring low-resistance levels to cefotaxime (AmpCs, TEM- and SHV-derived ESBLs). Our exclusion of other members of the *Enterobacterales* and the use of predominant colonies imply that the current results may underestimate the actual prevalence of intestinal colonization as well as the diversity in the gene pool of ESBLs, AmpCs, and carbapenemases in the Ghanaian community. There are considerable geographical differences in the occurrence of ESBL genotypes. Their distribution may vary across the country, and even within institutions [10,11]. Molecular testing detected the presence of bla genes in all isolates with clear ESBL phenotypes. It is worth noting that a wide array of other bla genes were not detected in this investigation. These include PER, IMP, and GES [63,64] which were not tested for because PCR was only carried out for some of the more common bla genes [10]. The questionnaire/interview aspect of the study also imposes certain limitations in terms of the quality of data for the risk factor analysis. In this case, the abstracted data of greatest concern include the history of antibiotic use. Because the antibiotic history was obtained by interviews and reviews of the drug repositories in respondents' households, certain received antibiotics could be missed.

5. Conclusion

In summary, we have shown that intestinal colonization with ESBLs (mostly CTX-M-15) is prevalent in Ghanaian communities and is one of the highest reported worldwide. Carbapenem antibiotics remain the most effective option against these isolates, however, the lonesome observation of the presence of NDM-1 metallo-beta-lactamases in intestinal flora of noninstitutionalized community persons remains a serious public health concern. The alarming spread of resistance genes should be stopped urgently by improving hygiene and streamlining the distribution and consumption of antibiotics. The importance of monitoring resistant bacteria in the future should include fecal sampling from the community.

Authors' contribution

N.ON., DS.H., MJ.N., N.FM., JA.P., KA.K., conceived the study; participated in its design, coordination, and collation of laboratory data. N.ON., GA.M., KN.B. performed laboratory work. N.ON. performed the statistical analysis. All authors helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2023.115918.

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