JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Prevalence and multidrug resistance of *Escherichia coli* from community-acquired infections in Lagos, Nigeria

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Abstract

Introduction: The emergence of multidrug resistance (MDR; resistance to ≥ 2 more antimicrobials) in *Escherichia coli* is of concern due to complications encountered in treatment.

Methodology: In this study, prevalence, antimicrobial resistance, and genetic characteristics of MDR community isolates of *E. coli* from Lagos, Nigeria were determined. Urine and stool samples were obtained from outpatients attending Lagos State hospitals and from animal handlers in abattoirs, poultries, and open markets, from December 2012 to July 2013.

Results: Approximately 50% of urine (200/394) and 88% of stool samples (120/136) were positive for *E. coli*. Based upon β-lactamase production, a subset of those isolates was selected for further study. Of the 22 antimicrobials tested, *E. coli* exhibited resistance to all antimicrobials except amikacin and piperacillin/tazobactam. The highest levels of resistance were to tetracycline (182/247; 73.7%), trimethoprim/sulfamethoxazole (152/247; 61.5%), and ampicillin (147/247; 59.1%). Resistance to the cephalosporins ranged from 1.6%–15% including the third- and fourth-generation cephalosporins, cefpodoxime (20/247; 8.1%) and cefepime (4/247; 1.6%), respectively. MDR was observed in 69.6% (172/247) of the isolates. Forty-eight *E. coli* resistant to at least five antimicrobials were selected for further analysis using pulsed-field gel electrophoresis; seven distinct clusters were observed among the diverse patterns. Of the 48 MDR *E. coli*, 30 different sequence types (ST) were detected using multilocus sequence typing, including four ST131.

Conclusions: This study demonstrated circulating MDR *E. coli* in the Nigerian community. Monitoring of antimicrobial resistance in developing countries is necessary to optimize empiric treatment and the prudent use of antimicrobials.

Key words: *E. coli*; antimicrobial resistance; humans; Nigeria.

J Infect Dev Ctries 2016; 10(9):920-931. doi:10.3855/jidc.7997

(Received 10 December 2015 – Accepted 09 March 2016)

Introduction

Antimicrobial drugs are important in the treatment of infections to reduce mortality and morbidity [1]. However, the emergence of antimicrobial resistance has posed a worldwide challenge to public health by hampering effective chemotherapy [2,3]. The use of antimicrobials in both human and veterinary medicine has led to the selection of resistant organisms, resulting in dissemination of drug-resistant bacteria in hospitals and the community [4,5]. The development of multidrug resistance (MDR; resistance to ≥ 2 antimicrobials) to major classes of antimicrobials within a short period of time after approval for medical use is cause for concern, especially due to the time required to produce new antimicrobial agents [6,7].

Escherichia coli, a normal inhabitant of the intestinal tract of mammals, is a common cause of intestinal infections, urinary tract infections (UTIs), and bacteremia in humans of all ages [8-11]. Resistance to a number of antimicrobials including aminoglycosides, β-lactams, cephalosporins, fluoroquinolones, sulfonamides, tetracycline, and trimethoprim have all been described in *E. coli* from clinical settings and food-producing animals [12-14]. MDR *E. coli* from the community have been described worldwide [15]. In the United States, prevalence of MDR *E. coli* among

community isolates increased more than 56% from the 1950s to the 2000s [1]. In Latin America, the widespread dissemination of phylogenetically related strains of uropathogenic *E. coli* in community and hospital settings has been observed and attributed to a common source in the community such as food and water or person-to-person transmission [16,17].

Many of the resistances in MDR *E. coli* are located on plasmids, which increases the possibility of clonal dissemination of these resistance classes in the community [18-21]. Spread of MDR *E. coli* globally can also be attributed to clones, such as *E. coli* sequence type 131 (ST131), known for its resistance to fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole, as well as its virulence and propensity to exchange genetic material, characteristics which further complicate therapy. A number of reports exist on the emergence of this clonal group in *E. coli* from the community and hospital infections in developed and developing countries [10,22,23]. In Nigeria, the emergence of *E. coli* ST131 and ST617 among clinical isolates of *E. coli* was reported in 2012 [21]. However, there is a paucity of data from community infections in Nigeria on the MDR profile of *E. coli* and their genetic lineages or reports on the major clonal complex circulating in the community. The potential spread of MDR *E. coli* is significant, especially for developing countries, which may have low financial resources for healthcare systems and poor infection control management. The antimicrobial resistance issue is exacerbated in these areas due to large numbers of the populace who tend to live in unhygienic conditions and have little access to healthcare and may be able to easily obtain antimicrobials, including counterfeit antimicrobials.

To provide more information on the extent of MDR *E. coli* in the human population in Nigeria, the prevalence and antimicrobial resistance of *E. coli* among outpatients and animal handlers in the community was determined. *E. coli* isolates in this study were tested against a wide range of broad-spectrum antimicrobials primarily used for treating human infections, including some of the most recently approved antimicrobials for human use. In addition, phylogenetic grouping, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE) were used to compare a subset of MDR *E. coli*.

Methodology

Bacterial isolates

Between December 2012 and July 2013, non-duplicate urine and stool samples were collected from asymptomatic patients at outpatient clinics in selected government hospitals in Lagos State and from animal handlers working in food production in the market and abattoirs in the State. Patients and animal handlers who were on antimicrobial therapy or had been on admission in any hospital six months prior to sampling were excluded from the study. Other requirements for participation in the study included urinary bacterial load of $\leq 10^5$ CFU/mL, no history of diarrhea in the preceding three months prior to the study, and willingness to respond to the questionnaire. Ethical approval for sampling was obtained from the Lagos State Health Service Commissions Board and Institutional Review Board of the hospitals. Informed consent was obtained from all human participants in this study.

All samples were collected in sterile universal bottles and kept on ice before processing; all were processed within 4 hours of collection. Stool samples were collected in modified Cary-Blair transport medium (Oxoid, Basingstoke, UK) and directly inoculated onto the agar plates. Urine and stool samples were cultivated on MacConkey agar and 5% sheep's blood agar (Oxoid, Basingstoke, UK) for the presence of members of the *Enterobacteriaceae* and incubated aerobically at 37°C for 18–24 hours. One to two colonies were selected from each sample. Isolates were Gram-stained and identified using API 20E according to the manufacturer's directions (bioMérieux, Basingstoke, UK). Colonies representative of *E. coli* were confirmed with the VITEK®2 System using the VITEK 2GN cards (bioMérieux, Durham, USA) according to the manufacturer's directions. A subset of β-lactamase-positive *E. coli* using the β-lactamase test strips (Oxoid, Basingstoke, UK) were used for the study. All bacterial strains were stored at -80°C in brain-heart infusion (BHI) broth containing 30% glycerol.

Antimicrobial susceptibility testing

Minimum inhibitory concentration (MIC, µg/mL) for *E. coli* was determined by broth microdilution using the Sensititre semi-automated susceptibility system (TREK Diagnostic Systems, Inc., Westlake, USA) and the Sensititre Gram-negative plate GN3F according to the manufacturer's directions. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) [24], with the exception of tigecycline and cephalothin. A breakpoint for resistance for tigecycline for *Enterobacteriaceae* has not been defined; for this study, the MIC for tigecycline was ≥ 1 µg mL^{-1}. The breakpoint for cephalothin as defined by

CLSI is ≥ 32 μg mL^{-1}; however, the highest concentration of cephalothin on the susceptibility plate was 16 μg mL^{1}. Therefore, the breakpoint used for determining resistance to cephalothin in this study was defined as > 16 μg mL^{-1}. Antimicrobials and breakpoints (in parenthesis) were as follows: amikacin (≥ 64 μg mL^{-1}), ampicillin (≥ 32 μg mL^{-1}), ampicillin/sulbactam (≥ 32/16 μg mL^{-1}), aztreonam (≥ 16 μg mL^{-1}), cefazolin (≥ 8 μg mL^{-1}), cefepime (≥ 32 μg mL^{-1}), cefoxitin (≥ 32 μg mL^{-1}), cefpodoxime (≥ 8 μg mL^{-1}), ceftazidime (≥ 16 μg mL^{-1}), ceftriaxone (≥ 4 μg mL^{-1}), cefuroxime (≥ 32 μg mL^{-1}), cephalothin (≥ 16 μg mL^{-1}), ciprofloxacin (≥ 4 μg mL^{-1}), ertapenem (≥ 2 μg mL^{-1}), gentamicin (≥ 16 μg mL^{-1}), meropenem (≥ 4 μg mL^{-1}), piperacillin/tazobactam (≥ 128/4 μg mL^{-1}), ticarcillin/clavulanic acid (≥ 128/2 μg mL^{-1}), tetracycline (≥ 16 μg mL^{-1}), ticarcillin/clavulanic acid (≥ 128/2 μg mL^{-1}), tobramycin (≥ 16 μg mL^{-1}), trimethoprim/sulfamethoxazole (≥ 4/76 μg mL^{-1}), and tigecycline (≥ 1 μg mL^{-1}). *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213 were controls for determination of MIC.

Phylogenetic analysis

Phylogenetic grouping of the eight phylo-groups for *Escherichia* (*i.e.*, A, B1, B2, C, D, E, F, and cryptic clade I) was performed using the extended quadruplex polymerase chain reaction (PCR) as previously described [25].

Multilocus sequence typing

MLST for seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) was performed as previously described [26]. Briefly, whole-cell template for PCR was prepared by suspending 2–3 bacterial colonies in 100 μL of sterile deionized water; 2 μL of template was used per reaction. Reaction mixtures (50 μL total) were composed of 2 μL of template DNA, 3 μL of each 10 mM primer (Eurofins Genomics, Huntsville, USA), 2.5 μL $MgCl_2$ (30 mM), 1 μL dNTP (2 mM) (Roche, Indianapolis, USA), and 0.5 μL *Taq* polymerase (5U/μL) (Roche); PCR products were amplified as previously described [26]. Products were purified for sequencing using the QIAGEN QIAquick PCR purification kit (QIAGEN, Valencia, USA) and sequenced using BigDye Ready Reaction Mix (Applied Biosystems, Foster City, USA) and the ABI Prism 3130x Genetic Analyzer (Applied Biosystems) according to the manufacturer's directions. Sequences were analyzed using BioNumerics software (Applied Maths Scientific Software Development, Sint-Martens-Latem, Belgium) and STs assigned using the *E. coli* MLST database (http://mlst.warwick.ac.uk/mlst/).

Pulsed-field gel electrophoresis

A 24-hour *E. coli* PFGE procedure was performed as previously described [27]. Briefly, cells from an overnight culture were embedded in 1.0% Seakem Gold agarose (BioWhittaker Molecular Applications, Rockland, USA) and digested with 10 U of *Xba*I (Roche Molecular Biochemicals, Indianapolis, USA). DNA standards were prepared from *Salmonella enterica* serotype Braenderup H9812. Digested DNA was separated using the CHEF-DRII PFGE system per the manufacturer's instructions (Bio-Rad, Hercules, USA). Electrophoresis was performed at 6 V for 19 hours with a ramped pulse time of 2.16–54.17 seconds in 0.5X Tris-borate-EDTA (TBE) at 14°C. Cluster analysis was determined with BioNumerics software using Dice coefficient and the unweighted pair group method (UPGMA). Optimization settings for dendrograms were 1.5% with a position tolerance of 1.5%. Isolates with ≥ 65% similar profiles were considered to represent the same clone [28].

Statistical analysis

Probability values of statistical significance were generated using Chi-square analysis (SAS version 9.1.3, SAS Institute Inc., Cary, USA). Statistical significance was defined as a probability value of less than or equal to 0.05 ($p \leq 0.05$).

Table 1. Prevalence of *Escherichia coli* among humans from the community.

Sample	No. of samples	No. of positive samples (%)	No. of *E. coli* isolates*
Urine			
Outpatient	334	180 (53.9)	131
Animal handler	60	20 (33.3)	11
Total	**394**	**200 (50.8)**	**142**
Stool			
Outpatient	116	112 (96.6)	98
Animal handler	20	8 (40)	7
Total	**136**	**120 (88.2)**	**105**

*Isolates selected for inclusion in this study were positive for β-lactamase production.

Results

Prevalence and antimicrobial resistance of E. coli

A total of 530 samples of human urine (n = 394) and stool (n = 136) were obtained from outpatients and from food animal handlers in the community. Of those, 50.8% (200/394) urine samples and approximately 88% of stool samples (120/136) were positive for *E. coli* (Table 1). Although the Gram-negative susceptibility plate used in this study contained 22 antimicrobials, the majority of those were β-lactams. Therefore, the *E. coli* selected for further study (n = 247) were those positive for β-lactamase production.

E. coli in this study were resistant to 20 of the 22 antimicrobials tested (Table 2). The highest level of resistance was to tetracycline (73.7%; 182/247) followed by trimethoprim-sulfamethoxazole (61.1%; 151/247) and ampicillin (59.1%; 146/247). Overall, the *E. coli* isolates exhibited resistance to all of the β-lactam antimicrobials tested except piperacillin-tazobactam. For the cephems, resistance was higher among the first-generation cephalosporins, cefazolin (15%; 37/247) and cephalothin (10.9%; 27/247). Resistance to second- and third-generation cephalosporins ranged from 2.4% to 8.9% for all isolates, while only 1.6% (4/247) exhibited resistance to the fourth-generation cephalosporin, cefepime (Table 2). Less than 2% of the isolates were resistant to the carbapenems, ertapenem (1.2%; 3/247) and meropenem (1.6%; 4/247). Of the three aminoglycosides tested, no resistance to amikacin was observed; however, isolates exhibited low resistance to gentamicin (7.7%; 19/247) and tobramycin (5.3%; 13/247) as well as to the glycylcycline antimicrobial, tigecycline (2%; 5/247). Nineteen percent of the isolates (47/247) were pan-susceptible.

Fifty-three resistance patterns were observed among all *E. coli* isolates (Table 3). Almost 70% of the isolates (172/247) were MDR, exhibiting resistance ranging from two to sixteen antimicrobials and two to five antimicrobial classes. Fifty-two isolates were resistant to five or more antimicrobials; two isolates that were resistant to sixteen antimicrobials collectively were resistant to the entire antimicrobial drug classes used in the study (Table 3). The largest groups of MDR, based upon different patterns of antimicrobials, belonged to those composed of three antimicrobials; fifteen combinational patterns were observed for this group. Surprisingly, nine different antimicrobial resistance combinations were observed for the groups composed of two antimicrobial classes and also five antimicrobial classes, suggesting wide-ranging MDR in the isolates. The three antimicrobials for which the *E. coli* isolates exhibited the highest level of resistance (ampicillin, tetracycline, and trimethoprim/sulfamethoxazole) also resulted in the most common resistance pattern, AmpTetSxt (n = 43) (Table 3).

Molecular characteristics

A subset of MDR *E. coli* (n = 48) resistant to five or more antimicrobials were selected for further analysis. Using the extended quadraplex *E. coli* phylo-typing method, five phylogenetic groups (A, B1, B2, C, and E) were identified; two isolates could not be assigned a phylo-group (unassigned; U) (Figure 1). Most of the isolates were identified as group B1 (n = 16), B2 (n = 11), or C (n = 11), while slightly fewer were group A (n = 7). Interestingly, the majority of MDR *E. coli* belonging to groups A, B1, and C were isolated from stool samples (n = 22) compared to urine samples (n = 12), while the majority of isolates from the virulent phylogenetic groups were isolated from urine samples (n = 10) rather than stool samples (n = 1). However, these results were not significantly different.

Among the 48 MDR *E. coli*, 30 STs were identified and frequently represented only once among the isolates (Figure 1). Two STs, ST410 (n = 5) and ST131 (n = 4), contained the greatest number of isolates; isolates belonging to ST410 were all related to commensal phylogenetic groups, while isolates identified as ST131 were also virulence associated.

Table 2. Antimicrobial resistance of *Escherichia coli* isolated from humans.

Antimicrobial*	Breakpoint (μg/mL)	No. resistant (%)
Ampicillin	≥ 32	146 (59.1)
Ampicillin/sulbactam	≥ 32/16	57 (23.1)
Aztreonam	≥ 16	19 (7.7)
Cefazolin	≥ 8	37 (15.0)
Cefepime	≥ 32	4 (1.6)
Ceftazidime	≥ 16	6 (2.4)
Cefoxitin	≥ 32	9 (3.6)
Cefpodoxime	≥ 8	20 (8.1)
Ceftriaxone	≥ 4	22 (8.9)
Cefuroxime	≥ 32	20 (8.1)
Cephalothin	≥ 16	27 (10.9)
Ciprofloxacin	≥ 4	32 (13.0)
Ertapenem	≥ 2	3 (1.2)
Gentamicin	≥ 16	19 (7.7)
Meropenem	≥ 4	4 (1.6)
Tetracycline	≥ 16	182 (73.7)
Ticarcillin/clavulanic acid	≥ 128/2	11 (4.5)
Tigecycline	≥ 1	5 (2.0)
Tobramycin	≥ 16	13 (5.3)
Trimethoprim/sulfamethoxazole	≥ 4/76	151 (61.1)

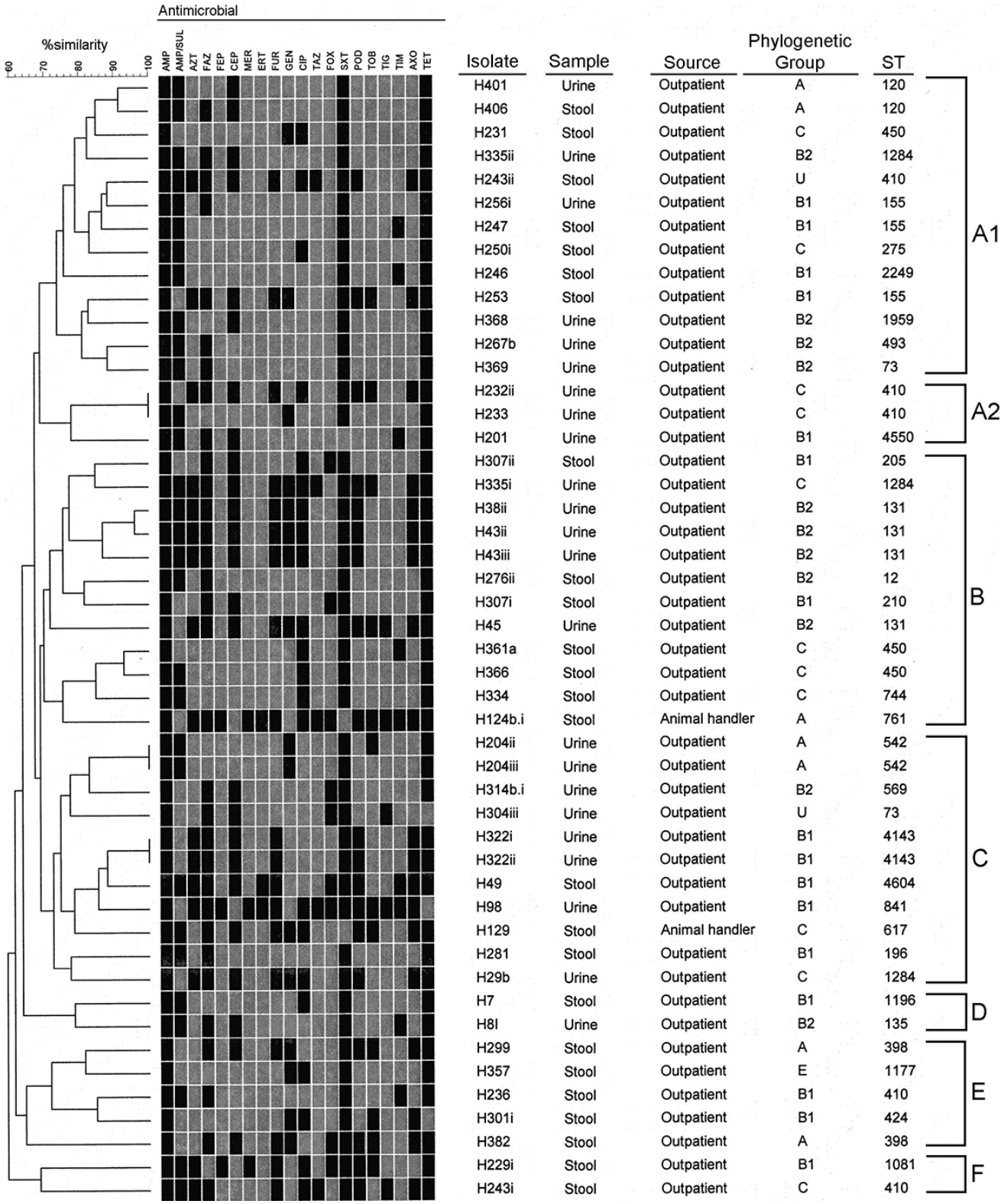
*No isolates were resistant to amikacin or piperacillin-tazobactam.

Table 3. Single and multidrug resistance patterns in *Escherichia coli* from humans.

Pattern	No. of resistances	No. of resistance by class	No. of isolates
Pan-susceptible	0	0	47
Amp	1	1	3
Azt	1	1	1
Cip	1	1	2
Faz	1	1	2
Sxt	1	1	3
Tet	1	1	17
AmpSxt	2	2	1
AmpTet	2	2	14
AxoSxt	2	2	1
AxoTet	2	2	1
FoxTet	2	2	1
TetSxt	2	2	23
AmpA/STet	3	2	3
AmpCipSxt	3	3	1
AmpFazTet	3	2	1
AmpTetSxt	3	3	43
CipTetSxt	3	3	1
FazTetTig	3	3	1
TetTigSxt	3	3	1
AmpA/STetSxt	4	3	20
AmpAztFepMer	4	1	1
AmpCipTetSxt	4	4	4
AmpGenTetSxt	4	4	2
AmpTetTigSxt	4	4	1
AmpA/SCepTetSxt	5	3	2
AmpA/SCipTetSxt	5	4	5
AmpA/SFazTetSxt	5	3	5
AmpA/SGenTetSxt	5	4	2
AmpA/STetTimSxt	5	3	4
AmpCipGenTetSxt	5	5	2
AmpCipTetTimSxt	5	4	1
AmpA/SFazCepTetSxt	6	3	2
AmpA/SFazCepTetTim	6	2	1
AmpA/SFazTetTimSxt	6	3	1
AmpA/SGenTetTobSxt	6	4	1
AmpAxoCipGenTobSxt	6	4	1
AmpFazFoxCepTetSxt	6	3	3
AmpA/SFazCepTetTimSxt	7	3	1
AmpFazFoxCepCipTetSxt	7	4	1
AmpAztFazPodAxoFurCepTetSxt	9	3	2
AmpFazPodAxoFurCepGenTetTobSxt	10	4	2
AmpA/SFazPodAxoFurCepCipGenTetTob	11	4	1
AmpAztFazPodAxoFurCepCipGenTetSxt	11	5	1
AmpAztFazPodAxoFurCepCipTetTobSxt	11	5	1
AmpAztFazPodAxoFurCepGenTetTobSxt	11	4	1
AmpA/SAztFazPodAxoFurCepCipGenTetSxt	12	5	3
AmpA/SAztFazPodTazAxoFurCepCipTetSxt	12	4	2
AmpA/SAztFepFoxPodFurCipMerTetTobSxt	12	5	1
AmpAztFazPodAxoFurCepCipGenTetTobSxt	12	5	1
AmpA/SAztFazFoxPodAxoFurCepErtTetTimSxt	13	3	1
AmpA/SAztFazPodTazAxoFurCepCipGenTetTobSxt	14	5	2
AmpAztFazFepFoxPodTazAxoFurCipErtMerTetTimTigTob	16	5	1
AmpAztFazFepFoxPodTazAxoFurCipErtMerTimTigTobSxt	16	5	1

Amp: ampicillin; A/S: ampicillin/sulbactam; Azt: aztreonam; Faz: cefazolin; Fep: cefepime; Taz: ceftazidime; Fox: cefoxitin; Pod: cefpodoxime; Axo: ceftriaxone; Fur: cefuroxime; Cep: cephalothin; Cip: ciprofloxacin; Ert: ertapenem; Gen: gentamicin; Mer: meropenem; Tet: tetracycline; Tim: ticarcillin/clavulanic acid; Tig: tigecycline; Tob: tobramycin; Sxt: trimethoprim/sulfamethoxazole; Pan-susceptible: susceptible to all tested antimicrobials.

Figure 1. Pulsed-field gel electrophoresis (PFGE) analysis, antimicrobial resistance patterns, phylogenetic groups and multilocus sequence typing (MLST) of multidrug-resistant (MDR; resistance to ≥ 2 antimicrobials) *E. coli* from humans. DNA for PFGE was digested with *Xba*I. Levels of similarity were determined using Dice coefficient and the unweighted pair group method (UPGMA). Clusters were based on ≥ 65% similarity and are labelled A–F. Black boxes represent resistance; gray boxes represent susceptible or intermediate. Antimicrobials are ampicillin (AMP), ampicillin/sulbactam (AMP/SUL), aztreonam (AZT), cefazolin (FAZ), cefepime (FEP), cephalothin (CEP), meropenem (MER), ertapenem (ERT), cefuroxime (FUR), gentamicin (GEN), ciprofloxacin (CIP), ceftazidime (TAZ), cefoxitin (FOX), trimethoprim/sulfamethoxazole (SXT), cefpodoxime (POD), tobramycin (TOB), tigecycline (TIG), ticarcillin/clavulanic acid (TIM), ceftriaxone (AXO), and tetracycline (TET). No isolates were resistant to amikacin or piperacillin-tazobactam.



Isolate	Sample	Source	Phylogenetic Group	ST	
H401	Urine	Outpatient	A	120	
H406	Stool	Outpatient	A	120	
H231	Stool	Outpatient	C	450	
H335ii	Urine	Outpatient	B2	1284	
H243ii	Stool	Outpatient	U	410	
H256i	Urine	Outpatient	B1	155	
H247	Stool	Outpatient	B1	155	
H250i	Stool	Outpatient	C	275	A1
H246	Stool	Outpatient	B1	2249	
H253	Stool	Outpatient	B1	155	
H368	Urine	Outpatient	B2	1959	
H267b	Urine	Outpatient	B2	493	
H369	Urine	Outpatient	B2	73	
H232ii	Urine	Outpatient	C	410	
H233	Urine	Outpatient	C	410	A2
H201	Urine	Outpatient	B1	4550	
H307ii	Stool	Outpatient	B1	205	
H335i	Urine	Outpatient	C	1284	
H38ii	Urine	Outpatient	B2	131	
H43ii	Urine	Outpatient	B2	131	
H43iii	Urine	Outpatient	B2	131	
H276ii	Stool	Outpatient	B2	12	
H307i	Stool	Outpatient	B1	210	B
H45	Urine	Outpatient	B2	131	
H361a	Stool	Outpatient	C	450	
H366	Stool	Outpatient	C	450	
H334	Stool	Outpatient	C	744	
H124b.i	Stool	Animal handler	A	761	
H204ii	Urine	Outpatient	A	542	
H204iii	Urine	Outpatient	A	542	
H314b.i	Urine	Outpatient	B2	569	
H304iii	Urine	Outpatient	U	73	
H322i	Urine	Outpatient	B1	4143	
H322ii	Urine	Outpatient	B1	4143	
H49	Stool	Outpatient	B1	4604	
H98	Urine	Outpatient	B1	841	C
H129	Stool	Animal handler	C	617	
H281	Stool	Outpatient	B1	196	
H29b	Urine	Outpatient	C	1284	
H7	Stool	Outpatient	B1	1196	
H8I	Urine	Outpatient	B2	135	D
H299	Stool	Outpatient	A	398	
H357	Stool	Outpatient	E	1177	
H236	Stool	Outpatient	B1	410	
H301i	Stool	Outpatient	B1	424	E
H382	Stool	Outpatient	A	398	
H229i	Stool	Outpatient	B1	1081	
H243i	Stool	Outpatient	C	410	F

Genetic relatedness

Using PFGE analysis, MDR *E. coli* isolates grouped into seven clusters (A–F) having ≥ 65% similarity (Figure 1). Clusters were examined to determine if they grouped according to PFGE pattern, antimicrobial resistance phenotype, source, phylogenetic group, or sequence type. Overall, the clusters appeared to contain isolates with a variety of different phenotypic and genotypic characteristics; however, source appeared to be a dominant character for a few clusters. Cluster A2 contained three isolates all from urine, and clusters E and F were composed of isolates all from stool samples (Figure 1). One of the larger clusters, cluster C, contained eleven isolates, mostly from urine samples. The other large clusters, A1 and B, were composed of almost even numbers of isolates from both urine and stool samples. STs represented by more than one isolate also appeared to cluster together, such as ST120 and ST155; however, that was not always consistently observed, as other STs (*e.g.*, ST1284, ST73) were located in different clusters (Figure 1).

Although not many isolates were identical, the three sets of isolates that had the same PFGE pattern (H232ii and H233; H204ii and H204iii; and H322i and H322ii) each belonged to the same phylogenetic group and the same ST. However, only isolates H322i and H322ii had identical antimicrobial resistance patterns (Figure 1). The four isolates identified as ST131 all grouped in cluster B and were resistant to twelve to thirteen antimicrobials, including antimicrobials in the aminoglycoside, fluoroquinolone, and sulfonamide classes. The two isolates resistant to sixteen antimicrobials (H124b.i and H98) were located in different clusters and had different STs, but were associated with the commensal *E. coli* phylogenetic group.

Discussion

E. coli is a leading cause of community and opportunistic infections in humans [29,30]. The increasing prevalence of MDR *E. coli* among community isolates is challenging because those isolates can occupy multiple niches, including human and animal hosts, thereby acquiring or transmitting antimicrobial resistance genes horizontally and vertically [31]. Earlier studies from clinical samples on antimicrobial resistance profiles of *E. coli* from Nigeria recorded a high prevalence to commonly administered antibiotics such as amoxicillin, amoxicillin-clavulanic acid, tetracycline, erythromycin, and trimethoprim/sulfamethoxazole [32-35]. However, few studies have assessed the antimicrobial and MDR profile of *E. coli* from community isolates in West Africa [36,37]. Most available data are specific to pathogenic *E. coli* isolates [13,38]. In this study, surveillance data was obtained on prevalence and MDR profiles of *E. coli* from outpatients and food-producing animal handlers in the community. The genetic lineages and the major clonal complexes of MDR *E. coli* circulating in the community were also investigated, which may be responsible for observed therapeutic failures [10]. This information is important for making informed decisions in the therapy of primary healthcare patients and forming strategies in the event of an outbreak of infection that could arise from these circulating clones in the community.

In this study, resistance to tetracycline was the most prevalent. This was not unexpected, as other studies from Nigeria have reported > 80% resistance to tetracycline [39,40]. Tetracycline is a commonly used antimicrobial in the Nigerian community, which may have given rise to the observed high resistance in this study. For MDR isolates, resistance to tetracycline, trimethoprim/sulfamethoxazole, and ampicillin was the predominant resistance phenotype. Similar findings on the resistance pattern of MDR of *E. coli* strains have been reported from Bangladesh and other parts of the world [41-45]. This may be attributed to the common use of these broad-spectrum antimicrobials for therapeutic purposes by clinicians in treating infections [35,38]. The sale of these drugs by medicine hawkers (non-professionals) in the Nigerian community has encouraged the practice of self-medication, leading to under- or over-dosage, which may also contribute to these observed resistance patterns [46]. More significantly, this study also indicated that most antibiotics used as first-choice oral empiric treatment are no longer appropriate since the prevalence of resistance exceeds 10% [47].

Cephalosporins are frequently prescribed by clinicians in Nigerian hospitals for treatment of bacterial infections [12,13], and studies on clinical isolates have shown high levels of resistance to cephalosporins [13,35]. In contrast to those studies, resistance to cephalosporins was low among *E. coli* isolates in this study. The non-phenotypic expression of resistance among isolates may be due to the study design, which excluded patients with previous intake of antimicrobial drugs within the previous six months of the study or admission into any healthcare facility.

The increasing rise in resistance to β-lactams, tetracyclines, and sulfa antimicrobials has led to the use of quinolones in difficult-to-treat infections and also in

the agricultural and veterinary sector [12,48-50]. This may have resulted in the recorded emergence of quinolone resistance in recent years. Nevertheless, this study tested only resistance to ciprofloxacin in the *E. coli* isolates, and it was observed that resistance to ciprofloxacin was reduced (less than 15%), which is similar to the conclusion of another study that reported that most of the uropathogens causing uncomplicated UTIs in outpatients are susceptible to fluoroquinolones [51,52]. This reduced resistance leading to low-level quinolone resistance can be attributed to various mechanisms, including reduced target expression or altered porins [53,54].

Carbapenem resistance exhibited by some MDR isolates in this study was unexpected because these antimicrobials are rarely used in Nigerian hospitals except in cases of emergencies or life-threatening conditions, primarily due to the high cost to purchase the drugs. These drugs are the last-line drugs in therapy of infections; resistance to carbapenems is an emerging concern in human medicine. In a study by Ogbolu *et al*. [55], carbapenem resistance with novel mechanisms of resistance was detected among Gram-negative bacterial isolates from hospital infections. No data exists on community isolates exhibiting resistance to carbapenems in Nigeria; however, studies have documented resistance to carbapenems in *Enterobacteriaceae* in other parts of the world among community isolates [56-59]. The presence of resistance to carbapenems in Lagos may be due to its geographical location, as Lagos is a border state with a high influx of people from surrounding African countries. In addition, easy access to imported frozen foods, which are cheaper than other meat sources, has been documented to be a major source of MDR bacteria in the country [60].

Resistance to broad-spectrum and easily available antibiotics (*i.e.*, ampicillin and trimethoprim/sulfamethoxazole) was high, similar to a report by Sahm *et al*. [61] that demonstrated dissemination of *E. coli* exhibiting resistance to broad-spectrum antibiotics among community isolates in the United States. Other drugs, such as tigecycline, are not easily available in Nigeria, which may account for the low level of resistance to tigecycline in *E. coli* isolates in this study. Nonetheless, the observed resistance, although low, may be due to the overexpression of efflux pumps or other mechanisms of resistance to new antibiotics existing before the drug was introduced into medical use [62,63]. The indiscriminate consumption of medicinal herbs by the community, which may contain a measure of natural antimicrobials, may also have contributed to the observed MDR among these isolates from the community [64]. Poor sanitation and overcrowding in the community may also be a significant risk factor in the dissemination of drug resistance among the isolates.

Genotyping of isolates has become important due to the possibility of an outbreak or horizontal transmission of certain bacterial strains. In the absence of whole genome sequencing, the discriminatory power and reproducibility has qualified PFGE as the gold standard in epidemiological studies for determining related strains from the same source [27]. PFGE analysis of the MDR *E. coli* revealed a genetically diverse population among human isolates. The majority of isolates belonging to the same cluster groups had at least 65% similarity, which is similar to a previous report by Nsofor *et al.* [65]. However, four pairs of isolates had 100% similarity, indicating that the isolates belonged to a single clonal group. One pair of identical isolates within the four groups was also resistant to the same antimicrobials. It is of note that some of the isolates that were in the same cluster had different antimicrobial phenotypes, suggesting that isolates with similar PFGE types do not necessarily have the same antimicrobial phenotypes because they may have acquired different antibiotic resistance genes and unique DNA rearrangements [21,66,67].

Genetic analysis of the isolates also determined common genetic lineages among isolates, and diverse STs were identified. This study confirmed the presence of 30 STs for *E. coli* belonging to phylogenetic groups A, B1, B2, C, and E circulating in the community. Commensal *E. coli* typically belong to group A, B1, or C, while isolates from phylogenetic group B are associated with virulence and account for the majority of extra-intestinal infections [68,69]. Phylogenetic group B isolates have also been associated with avian phylogenetic groups, which pose a potential zoonotic risk [70-72]. Dissemination of these clones to humans through the food chain is a possibility since Lagosians depend on food-producing animals, especially poultry, as a main source of protein [14,72]. Phylogenetic group A has been reported to be more prevalent within the United States than abroad and infects urinary tract and non-urinary tract sites [73]. These strains have also been associated with trimethoprim-sulfamethoxazole resistance, which was also observed in this study. Phylogenetic group C has only been recognized in the last few years and is closely related to phylogenetic group B1 [25]. A recent study has shown that isolates belonging to this group possess traits such as colonization and ability to disseminate in the intestinal

tract, which may increase the pathogenicity of these isolates [74].

Little data exists on the presence of ST131 and other clonal groups in Africa. *E. coli* ST131 virulence-associated clonal group was detected in the present study with similar MDR patterns, suggesting the possibility of clonal spread of these MDR *E. coli*. The presence of *E. coli* ST131 isolates in West Africa was reported by Aibinu *et al*. [21] from hospital infections in Lagos, while one other *E. coli* ST131 was identified from a study of inpatients from Oyo State, Nigeria [36]. In a report from another African country, approximately 45% of extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates were ST131, which suggested a high rate of virulence-associated *E. coli* in circulation in Cape Town [75]. Travel to Africa has also been indicated as a factor in *E. coli* ST131 travel-related ESBL-producing *E. coli* infections [76]. These clones threaten public health due to their virulence factors, rapid dissemination, and the lack of new drugs to combat the spread [23].

Conclusions

An urgent need for antimicrobial surveillance in the public health sector is of utmost importance; this study provides needed data from the community to assist in this effort. The declining rate of production of new antimicrobials and an increase in MDR pathogens in the clinic and community is precipitating a global health crisis [77]. Continuous migration and international travel have led to increased antimicrobial resistance in this group of bacteria due to mobile genetic elements that are easily spread through direct contact and ingestion of contaminated water or food [29,78,79]. It is therefore necessary to screen outpatients for asymptomatic bacterial infections and also food animal handlers before they handle animal food products sold to the community to prevent transmission or circulation of clonal strains of MDR *E. coli*.

References

1. Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew MJ, McDermott PF (2012) Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. Emerg Infect Dis 18: 741-749.
2. Bradford PA (2001) What's new in beta-lactamases? Curr Infect Dis Rep 3: 13-19.
3. Naseer U, Sundsfjord A (2011) The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. Microb Drug Resist 17: 83-97.
4. Wenzel RP, Bearman G, Edmond MB (2008) Screening for MRSA: a flawed hospital infection control intervention. Infect Control Hosp Epidemiol 29: 1012-1018.
5. Szmolka A, Nagy B (2013) Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Front Microbiol 4: 1-13.
6. Frye JG, Jackson CR (2013) Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica*, *Escherichia coli*, and *Enterococcus* spp. isolated from U.S. food animals. Front Microbiol 4: 1-22.
7. Walsh C (2003) Where will new antibiotics come from? Nat Rev Microbiol 1: 65-70.
8. Ruppe E, Hem S, Lath S, Gautier V, Ariey F, Sarthou JL, Monchy D, Arlet G (2009) CTX-M beta-lactamases in *Escherichia coli* from community-acquired urinary tract infections, Cambodia. Emerg Infect Dis 15: 741-748.
9. Ruppe E, Woerther PL, Diop A, Sene AM, Da CA, Arlet G, Andremont A, Rouveix B (2009) Carriage of CTX-M-15-producing *Escherichia coli* isolates among children living in a remote village in Senegal. Antimicrob Agents Chemother 53: 3135-3137.
10. Rogers BA, Sidjabat HE, Paterson DL (2011) *Escherichia col*i O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother 66: 1-14.
11. Laupland KB (2013) Incidence of bloodstream infection: a review of population-based studies. Clin Microbiol Infect 19: 492-500.
12. Aibinu I, Adenipekun E, Odugbemi T (2004) Emergence of quinolone resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State hospitals in Nigeria. Nigerian J Health Biomed Sci 3: 73-78.
13. Ogbolu DO, Daini OA, Ogunledun A, Alli AO, Webber MA (2011) High levels of multidrug resistance in clinical isolates of Gram-negative pathogens from Nigeria. Int J Antimicrob Agents 37: 62-66.
14. Adenipekun EO, Jackson CR, Oluwadun A, Iwalokun BA, Frye JG, Barrett JB, Hiott LM, Woodley TA (2015) Prevalence and antimicrobial resistance in *Escherichia coli* from food animals in Lagos, Nigeria. Microb Drug Resist 21: 358-365.
15. Lynch JP, III, Clark NM, Zhanel GG (2013) Evolution of antimicrobial resistance among *Enterobacteriaceae* (focus on extended spectrum beta-lactamases and carbapenemases). Expert Opin Pharmacother 14: 199-210.
16. Dias RC, Marangoni DV, Smith SP, Alves EM, Pellegrino FL, Riley LW, Moreira BM (2009) Clonal composition of *Escherichia coli* causing community-acquired urinary tract infections in the State of Rio de Janeiro, Brazil. Microb Drug Resist 15: 303-308.
17. Dias RC, Marangoni DV, Riley LW, Moreira BM (2009) Identification of uropathogenic *Escherichia coli* clonal group A (CgA) in hospitalised patients. Mem Inst Oswaldo Cruz 104: 787-789.

18. van der Donk CF, van de Bovenkamp JH, De Brauwer EI, De MP, Feldhoff KH, Kalka-Moll WM, Nys S, Thoelen I, Trienekens TA, Stobberingh EE (2012) Antimicrobial resistance and spread of multi drug resistant *Escherichia coli* isolates collected from nine urology services in the Euregion Meuse-Rhine. PLoS ONE 7: 1483-1489.
19. Chen X, He L, Li Y, Zeng Z, Deng Y, Liu Y, Liu JH (2014) Complete sequence of a F2:A-:B- plasmid pHN3A11 carrying rmtB and qepA, and its dissemination in China. Vet Microbiol 174: 267-271.
20. Williamson DA, Lim A, Wiles S, Roberts SA, Freeman JT (2013) Population-based incidence and comparative demographics of community-associated and healthcare-associated *Escherichia coli* bloodstream infection in Auckland, New Zealand, 2005-2011. BMC Infect Dis 13: 385-392.
21. Aibinu I, Odugbemi T, Koenig W, Ghebremedhin B (2012) Sequence type ST131 and ST10 complex (ST617) predominant among CTX-M-15-producing *Escherichia coli* isolates from Nigeria. Clin Microbiol Infect 18: E49-E51.
22. Lau SH, Kaufmann ME, Livermore DM, Woodford N, Willshaw GA, Cheasty T, Stamper K, Reddy S, Cheesbrough J, Bolton FJ, Fox AJ, Upton M (2008) UK epidemic *Escherichia coli* strains A-E, with CTX-M-15 beta-lactamase, all belong to the international O25:H4-ST131 clone. J Antimicrob Chemother 62: 1241-1244.
23. Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, Phan MD, Gomes MD, Peters KM, Davies M, Rogers BA, Dougan G, Rodriguez-Bano J, Pascual A, Pitout JD, Upton M, Paterson DL, Walsh TR, Schembri MA, Beatson SA (2014) Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci U S A 111: 5694-5699.
24. Clinical and Laboratory Standards Institute (2014) M100-S24 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI: Wayne, PA.
25. Clermont O, Christenson JK, Denamur E, Gordon DM (2013) The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5: 58-65.
26. Tartof SY, Solberg OD, Manges AR, Riley LW (2005) Analysis of a uropathogenic *Escherichia coli* clonal group by multilocus sequence typing. J Clin Microbiol 43: 5860-5864.
27. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ (2006) Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 3: 59-67.
28. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33: 2233-2239.
29. Allocati N, Masulli M, Alexeyev MF, Di IC (2013) *Escherichia coli* in Europe: an overview. Int J Environ Res Public Health 10: 6235-6254.
30. Pitout JD (2010) The latest threat in the war on antimicrobial resistance. Lancet Infect Dis 10: 578-579.
31. Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10: S122-S129.
32. Motayo BO, Ogiogwa I, Okerentugba PO, Innocent-Adiele HC, Nwanze JC, Onoh CC, Okonko IO (2012) Antimicrobial resistance profile of extra-intestinal *Escherichia coli* infections in a southwestern Nigerian city. J Microbiol Res 2: 141-144.
33. Olukoya DK, Daini O, Alabi SA, Coker AO, Odugbemi T, Akinrimisi EO (1988) Antimicrobial resistance patterns and plasmids of enteropathogenic *Escherichia coli* isolated in Nigeria. Eur J Epidemiol 4: 306-309.
34. Okeke IN, Aboderin OA, Byarugaba DK, Ojo KK, Opintan JA (2007) Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg Infect Dis 13: 1640-1646.
35. Olowe OA, Grobbel M, Buchter B, Lubke-Becker A, Fruth A, Wieler LH (2010) Detection of *bla*(CTX-M-15) extended-spectrum beta-lactamase genes in *Escherichia coli* from hospital patients in Nigeria. Int J Antimicrob Agents 35: 206-207.
36. Inwezerua C, Mendonca N, Calhau V, Domingues S, Adeleke OE, Da Silva GJ (2014) Occurrence of extended-spectrum beta-lactamases in human and bovine isolates of *Escherichia coli* from Oyo state, Nigeria. J Infect Dev Ctries 8: 774-779.
37. Namboodiri SS, Opintan JA, Lijek RS, Newman MJ, Okeke IN (2011) Quinolone resistance in *Escherichia coli* from Accra, Ghana. BMC Microbiol 11: 44-52.
38. Aibinu IE, Pfeifer Y, Ogunsola F, Odugbemi T, Koenig W, Ghebremedhin B (2011) Emergence of beta-lactamases OXA-10, VEB-1 and CMY in *Providencia* spp. from Nigeria. J Antimicrob Chemother 66: 1931-1932.
39. Okesola AO, Aroundegbe TI (2011) Antibiotic resistance pattern of uropathogenic *Escherichia coli* in South West Nigeria. Afr J Med Med Sci 40: 235-238.
40. Aboderin OA, Abdu AR, Odetoyin BW, Lamikanra A (2009) Antimicrobial resistance in *Escherichia coli* strains from urinary tract infections. J Natl Med Assoc 101: 1268-1273.
41. Davis MA, Lim JY, Soyer Y, Harbottle H, Chang YF, New D, Orfe LH, Besser TE, Call DR (2010) Development and validation of a resistance and virulence gene microarray targeting *Escherichia coli* and *Salmonella enterica*. J Microbiol Methods 82: 36-41.
42. Mowla R, Imam KM, Asaduzzaman M, Nasrin N, Raihan SZ, Chowdhury AK (2011) Emergence of multidrug resistant Extended-Spectrum Beta-Lactamase producing *Escherichia coli* associated with urinary tract infections in Bangladesh. J Basic Clin Pharm 3: 225-228.
43. Xiao YH, Wang J, Li Y (2008) Bacterial resistance surveillance in China: a report from Mohnarin 2004-2005. Eur J Clin Microbiol Infect Dis 27: 697-708.
44. Guerra B, Junker E, Schroeter A, Malorny B, Lehmann S, Helmuth R (2003) Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. J Antimicrob Chemother 52: 489-492.
45. Khan A, Das SC, Ramamurthy T, Sikdar A, Khanam J, Yamasaki S, Takeda Y, Nair GB (2002) Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. J Clin Microbiol 40: 2009-2015.
46. Salako LA, Brieger WR, Afolabi BM, Umeh RE, Agomo PU, Asa S, Adeneye AK, Nwankwo BO, Akinlade CO (2001) Treatment of childhood fevers and other illnesses in three rural Nigerian communities. J Trop Pediatr 47: 230-238.
47. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AJ, Soper DE (2011) International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of

America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 52: e103-e120.
48. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18: 657-686.
49. Paterson DL (2006) Resistance in gram-negative bacteria: *Enterobacteriaceae*. Am J Infect Control 34: S20-S28.
50. Fortini D, Fashae K, Garcia-Fernandez A, Villa L, Carattoli A (2011) Plasmid-mediated quinolone resistance and beta-lactamases in *Escherichia coli* from healthy animals from Nigeria. J Antimicrob Chemother 66: 1269-1272.
51. Dalhoff A (2012) Global fluoroquinolone resistance epidemiology and implications for clinical use. Interdiscip Perspect Infect Dis 2012: 1-37.
52. Dalhoff A (2012) Resistance surveillance studies: a multifaceted problem--the fluoroquinolone example. Infection 40: 239-262.
53. Ince D, Hooper DC (2003) Quinolone resistance due to reduced target enzyme expression. J Bacteriol 185: 6883-6892.
54. Chapman JS, Bertasso A, Cummings LM, Georgopapadakou NH (1995) Low-level resistance to the cephalosporin 3'-quinolone ester Ro 23-9424 in *Escherichia coli*. Antimicrob Agents Chemother 39: 564-566.
55. Ogbolu DO, Webber MA (2014) High-level and novel mechanisms of carbapenem resistance in Gram-negative bacteria from tertiary hospitals in Nigeria. Int J Antimicrob Agents 43: 412-417.
56. Moquet O, Bouchiat C, Kinana A, Seck A, Arouna O, Bercion R, Breurec S, Garin B (2011) Class D OXA-48 carbapenemase in multidrug-resistant enterobacteria, Senegal. Emerg Infect Dis 17: 143-144.
57. Aubron C, Poirel L, Fortineau N, Nicolas P, Collet L, Nordmann P (2005) Nosocomial spread of *Pseudomonas aeruginosa* isolates expressing the metallo-beta-lactamase VIM-2 in a hematology unit of a French hospital. Microb Drug Resist 11: 254-259.
58. Aubron C, Poirel L, Ash RJ, Nordmann P (2005) Carbapenemase-producing *Enterobacteriaceae*, U.S. rivers. Emerg Infect Dis 11: 260-264.
59. Nordmann P, Poirel L, Toleman MA, Walsh TR (2011) Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother 66: 689-692.
60. Adeyanju GT, Ishola O (2014) *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Springerplus 3: 139.
61. Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA (2001) Multidrug-resistant urinary tract isolates of *Escherichia coli*: prevalence and patient demographics in the United States in 2000. Antimicrob Agents Chemother 45: 1402-1406.
62. Levy SB (2002) Antimicrobial consumer products: where's the benefit? What's the risk? Arch Dermatol 138: 1087-1088.
63. Hawkey P, Finch R (2007) Tigecycline: in-vitro performance as a predictor of clinical efficacy. Clin Microbiol Infect 13: 354-362.
64. Oreagba IA, Oshikoya KA, Amachree M (2011) Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complement Altern Med 11: 117-125.
65. Nsofor CA, Iroegbu CU, Call DR, Davies MA (2013) The genetic relatedness of drug resistant *E. coli* isolates of human and animal origin in Nigeria. Int J Genet Mol Biol 5: 37-41.
66. Beutin L, Kaulfuss S, Herold S, Oswald E, Schmidt H (2005) Genetic analysis of enteropathogenic and enterohemorrhagic *Escherichia coli* serogroup O103 strains by molecular typing of virulence and housekeeping genes and pulsed-field gel electrophoresis. J Clin Microbiol 43: 1552-1563.
67. Loli A, Tzouvelekis LS, Tzelepi E, Carattoli A, Vatopoulos AC, Tassios PT, Miriagou V (2006) Sources of diversity of carbapenem resistance levels in *Klebsiella pneumoniae* carrying *bla*VIM-1. J Antimicrob Chemother 58: 669-672.
68. Clermont O, Bonacorsi S, Bingen E (2000) Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66: 4555-4558.
69. Branger C, Zamfir O, Geoffroy S, Laurans G, Arlet G, Thien HV, Gouriou S, Picard B, Denamur E (2005) Genetic background of *Escherichia coli* and extended-spectrum beta-lactamase type. Emerg Infect Dis 11: 54-61.
70. Russo TA, Johnson JR (2003) Medical and economic impact of extraintestinal infections due to *Escherichia coli* focus on an increasingly important endemic problem. Microbes Infect 5: 449-456.
71. Hiki M, Usui M, Akiyama T, Kawanishi M, Tsuyuki M, Imamura S, Sekiguchi H, Kojima A, Asai T (2014) Phylogenetic grouping, epidemiological typing, analysis of virulence genes, and antimicrobial susceptibility of *Escherichia coli* isolated from healthy broilers in Japan. Ir Vet J 67: 14-18.
72. Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, Reid-Smith RJ, Zhanel GG, Manges AR (2012) Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. Emerg Infect Dis 18: 415-421.
73. Johnson JR, Murray AC, Kuskowski MA, Schubert S, Prere MF, Picard B, Colodner R, Raz R (2005) Distribution and characteristics of *Escherichia coli* clonal group A. Emerg Infect Dis 11: 141-145.
74. Moissenet D, Salauze B, Clermont O, Bingen E, Arlet G, Denamur E, Merens A, Mitanchez D, Vu-Thien H (2010) Meningitis caused by *Escherichia coli* producing TEM-52 extended-spectrum beta-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. J Clin Microbiol 48: 2459-2463.
75. Peirano G, van Greune CH, Pitout JD (2011) Characteristics of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* from community hospitals in South Africa. Diagn Microbiol Infect Dis 69: 449-453.
76. Pitout JD, Campbell L, Church DL, Gregson DB, Laupland KB (2009) Molecular characteristics of travel-related extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates from the Calgary Health Region. Antimicrob Agents Chemother 53: 2539-2543.
77. Dantas G, Sommer MO (2012) Context matters - the complex interplay between resistome genotypes and resistance phenotypes. Curr Opin Microbiol 15: 577-582.
78. Orsi RH, Stoppe NC, Sato MI, Ottoboni LM (2007) Identification of *Escherichia coli* from groups A, B1, B2 and D in drinking water in Brazil. J Water Health 5: 323-327.
79. Orsi RH, Stoppe NC, Sato MI, Gomes TA, Prado PI, Manfio GP, Ottoboni LM (2007) Genetic variability and pathogenicity potential of *Escherichia coli* isolated from recreational water reservoirs. Res Microbiol 158: 420-427.

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Conflict of interests: No conflict of interests is declared.

Disclosure Statement: The mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.