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Cluster analysis and geospatial mapping of antibiotic resistant *Escherichia coli* O157 in southwest Nigerian communities

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ABSTRACT

Geospatial spread and antibiotic-resistant relatedness of *Escherichia coli* O157, which are important virulent serotypes causing severe complications leading to high intestinal morbidity and occasional mortality in several communities in southwest Nigeria, were evaluated.

Biotyped *Escherichia coli* strains ($n = 508$) from subjects with diarrhea and related intestinal infections, various domestic water sources and food animal products were evaluated for antibiotic resistance relatedness, conjugative activity, virulence factor and biofilm production. Antibiotic resistance of *Escherichia coli* O157 encoded with *stx* was mapped for geospatial spread.

Detected stx-encoded *Escherichia coli* O157 (7.56%) of human strains were significantly higher compared to water and food animal strains ($p = 0.001$) with high conjugative and transformative activity (OR(95%CI) = 34.65(94.5); $p = 0.023$). Water- *Escherichia coli* O157 reveal significant median resistance to ciprofloxacin, gentamycin ($p < 0.05$) and human diarrheagenic strains showed >60% resistance to doxycycline (MIC_{50} 8 μg/mL and MIC_{90} 128 μg/mL; $p = 0.018$), tetracycline (MIC_{50} 4 μg/mL and MIC_{90} 64 μg/mL), ciprofloxacin (MIC_{50} 2 μg/mL and MIC_{90} 128 μg/mL) and gentamycin (MIC_{50} 4 μg/mL and MIC_{90} 256 μg/mL). Strains from human diarrhea, UTI, colitis, cattle, fish, sheep, ground waters, streams, and rivers characterized with biofilm, hemolysin, protease productions, R-plasmid (≈14.30kbp) and MARI (0.84) were highly related. Principal component analysis (score plot) revealed a significant association between resistant human diarrheic strains with cattle and poultry strains. A high population of heterogeneous *stx*-encoded diarrheagenic and colitis strains was predominant in urban settings spreading with food animal and water *Escherichia coli* O157 strains.

Human diarrheagenic *Escherichia coli* O157 were highly related to antibiotic resistance and virulence pattern with water and animal products strains. Strategic interventions through the implementation of One Health approach and population-target antimicrobial stewardship are needed to mitigate the increasing intestinal morbidity and reduction of mortality impact. Regular application of spatial data on clonal dissemination is important for monitoring, surveillance of antimicrobial resistance and transmission of zoonotic food-borne *Escherichia coli* O157 pathogens.

1. Introduction

Community spread and poor detection of *Escherichia coli* O157-producing shiga toxin (*stx*), characterized with antibiotic resistance intensify the disease burden associated with hemorrhagic colitis and threatening systemic infections [1]. Production of shiga toxin and other virulent factors enhances severe clinical manifestations involving vascular endothelial cells, resulting in vasculitis, hemolytic uremic syndrome, abdominal pain, and rarely, thrombotic thrombocytopenic purpura [2,3]. Shiga toxin producing *Escherichia coli* O157 mostly

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initiates an inflammatory cascade leading to leukocyte aggregation, apoptosis, platelet aggregation, microthrombic formation, hemolysis, renal dysfunction and diffuse vasculitic injury resulting to multiple organ failures [4].

Continuous dissemination of *Escherichia coli* O157 strains in communities of sub-Sahara Africa has been linked to several risk factors including behavioral attitudes, demographic changes, environmental and food safety, decrease economic output and declining health status causing huge morbidity and occasional death. Poor surveillance and continuous spread further contribute to re-infection, increase antibiotic resistance, and population infection risk [5].

Contamination of various water sources used for domestic purposes, particularly nearby surface water (such as rivers and streams) with channels flowing through ruminant abattoirs [6], poultry farms [7]; and nearby groundwater (e.g well or bore-hole) to sewage run-off [8] subjecting the populace to *Escherichia coli* O157 infections. There is increasing undetected contamination of *Escherichia coli* O157 in food animal products frequently consumed in several Nigerian communities [5], making the disease burden taking a highly devastating effect on public health [9–11]. Reports from African countries showed an incidence rate of 1.4 cases per 100,000 people yearly [12], and this contributes to increase burden of *Escherichia coli* O157 identified in retailed meat products from cattle, sheep, goat, pig [12,13], sachet water in Nigeria [14], Ghana [15], Uganda [16], Cote d'Ivoire and Burkina Faso [17]. Prevalence of hemolytic strains with multidrug-resistant pattern (MDR) reported among various age groups with particular emphasis on virulent shiga toxigenic serotypes remain public health concern in many African countries [9]. From reported cases in Africa, there are limited geospatial data on the prevalent multi-drug resistant *Escherichia coli* O157 pathotypes, with clonal relatedness to enterohemorrhagic *Escherichia coli* pathotypes from water and animal products suspected to cause infection in human subjects [11].

Unregulated antibiotic use in animal feed, particularly in ruminants, treatment of infection and water pollution, drives the increasing antibiotic resistance. Neglect of *Escherichia coli* O157 detection and surveillance in diarrheagenic or renal infections further increase misdiagnosis, inadequate treatment, and intestinal morbidity rates. Application of One Health strategy and assessment of antibiotic-resistant cluster relatedness of prevalent animal and water *Escherichia coli* O157 strains with human infections provide better insight into the transmission, tracking, and epidemiology for effective prevention. The use of the Global Positioning System (GPS) for antimicrobial surveillance, collection of relevant health data, and monitoring prevalent *Escherichia coli* O157 is poorly utilized in southwest Nigeria. Therefore, the study evaluates the antibiotic resistance relatedness and geospatial spread of the human *Escherichia coli* O157 with food animal and water strains in select southwest Nigerian communities.

2. Methods

2.1. Ethical statement

Approval for clinical strain collection was obtained from the Ethical Board of Federal Medical Centre, Abeokuta (FMCA/470/HREC/11/2018; NHREC/08/10–2015), and the consent of the owners of groundwater (well and bore-hole water) was sought. The food animal products (meats, visceral organs etc) were purchased from wholesalers and/or retailers offering these items for sale at various popular markets.

2.2. Sampling

A total of 508 *Escherichia coli* strains were isolated between June 2018 and December 2019 from subjects (n = 227) with suspected diarrheic and related intestinal infection from several communities in southwest Nigeria attending the In- and Out-patient clinics of the Federal Medical Centre, Abeokuta Nigeria. Water samples from streams, rivers, wells and domestic reservoirs (n = 127) and retailed food animal products such as meat, skin and visceral organs from sheep, cattle, goat and poultry (n = 154) from select locations in southwest Nigeria were collected for the study. Collected human and food animal samples were cultured on MacConkey, Eosin Methylene Blue and Sorbitol MacConkey agars; incubated at 37 °C while water samples were pre-enriched in Nutrient glucose broth for 48 h before sub-culturing on Sorbitol-MacConkey and Eosine methylene blue agars. The pure cultures of suspected *Escherichia coli* strains were biotyped and the population showing non-sorbitol fermentation were serotyped with Latex agglutination test kit DR0620M (Oxoid LTD Hampshire, England) to classify the strains to O157 serogroup as described by Nataro and Kaper [18].

2.3. Stx1 and stx2 genotyping.

Detected Latex *E. coli* O157-positive strains were selected for *stx1* and *stx2* genotyping. Extracted chromosomal DNA from overnight pure broth culture was obtained using a commercial kit following the manufacturer's instruction. Amplification for *stx1* was carried out in a reaction volume of 20 μL containing 10 μL 2× MyTaq HS Mix (10 μL), 1 μL each of *stx1* (2.5 μM) forward primer (5′-ACACTGGATGATCTCAGTGG-3′) and reverse primer (5′-CTGAATCCCCCTCCTTATG-3′), 2.0 μL of extracted chromosomal DNA and makeup with nuclease-free water. For *stx2* amplification, 20 μL final volume containing 1 μL each of *stx2* (2.5 μM) forward primer (5′-CCATGACAACGGACAGCAGTT-3′) and reverse primer (5′-CCTGTCAACCGGTGAGCGCACTTTC-3′), 2.0 μL of chromosomal DNA, 10 μL 2× MyTaq HS Mix (10 μL) and makeup with nuclease-free water. The amplification reaction for *stx1* and *stx2* was carried out in 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 60 s as previously described [5,19]. Obtained DNA amplicons were electrophoresed on 1.5% agarose containing Ethidium bromide (10 mg/mL) at a current of 100 V for 30 min with a standard marker (1 kb) and then viewed using ultraviolet photo-documentation to evaluate carriage of *stx1* and *stx2*.

2.4. Antibiogram

Latex serotyped *E. coli* O157 strains from human, food animal and water sources were profiled for phenotypic antibiotic resistance to select antibiotics frequently used for the treatment of human enteric and urinary infections and as animal feed-additives. Standard operational procedures of the Kirby Bauer disk diffusion method [20] were used for phenotypic resistance assay of nitrofurantoin (300 μg), gentamicin (10 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), ofloxacin and amoxicillin (10 μg) against the isolates. Values were interpreted with CLSI [21] and the assay included a reference strain *E. coli* (ATCC 25922) as the control strain. Commonly prescribed antibiotics for the clinical therapy and management of intestinal and extra-intestinal infections (amoxicillin-clavulanic acid, nitrofurantoin, ciprofloxacin, ceftazidime, cefuroxime, gentamycin, doxycycline, ofloxacin and amoxicillin) were evaluated for minimum inhibitory concentration (MIC) at dilution ranges of 0.5–128 μg/mL using broth micro-dilution method. The MIC_{50} and MIC_{90} values of each antibiotic were calculated as the lowest concentration of the antibiotic at which 50% and 90% of the isolates were inhibited respectively [22]. The multi-antibiotic resistance index (MARI) was calculated for each strain to determine their resistance level according to their sources [19].

2.5. R-plasmid profiling and mating analysis

The carriage of extra-chromosomal resistance plasmid (R-Plasmid) among the resistant *Escherichia coli* O157 was investigated using the Alkaline lysis method [23] and further profiled for molecular weight using photo-documentation for evaluation of DNA band sizes. The ability of the R-plasmid DNA to be transferred and transform non-*Escherichia coli* O157 competent cells to resistance strain was determined

by conjugation [24]. Identified wild R-plasmid encoded *Escherichia coli* O157 from human subjects, water and animal products were transconjugated with susceptible *Escherichia coli* recipient strains (ATCC 25567, amp^S, tet^S, $gent^S$, cfz^S, lactose fermenter, sorbitol non-fermenter strain) 1:10 ratio (donor to recipient) in 0.5% sucrose-enriched Luria-Bertani broth at 37 °C with constant shaking. After 3 h, it was then sub-cultured onto Sorbitol-MacConkey agar plates supplemented with 6 mg/mL Penicillin and ceftazidime (Oxoid, UK). Recovered transconjugants showing non-Sorbitol fermentation and non-O157 lactose fermented strains were profiled for R-plasmid with control strains. For transformation activity, extracted R-plasmid DNA was transferred into non-O157 *Escherichia coli* recipients. Briefly, 20 μL extracted plasmid DNA obtained from each *Escherichia coli* O157 isolate was added to separate 5 mL overnight enriched Luria-Bertani broth culture containing susceptible *E. coli* recipient strains ATCC 25567 strains (amp^S, tet^S, $gent^S$, cfz^S, lactose fermenter, sorbitol non-fermenter strain) and 2 M calcium chloride. After 6 h of incubation at 37 °C, the mixture was sub-cultured on a selection plate of Sorbitol-MacConkey agar supplemented with 4 mg/L ampicillin and ceftazidime and further incubated at 37 °C for 18–24 h. Selected transformants growing on the selection plates were evaluated for antimicrobial susceptibility and assayed for R-plasmid as described [24], with slight modification to ascertain possible transformation of the susceptible *E. coli* recipients.

2.6. Biofilm production

Each identified *Escherichia coli* O157 strain was assessed for biofilm production on flat-bottom polystyrene microtiter plates at 37 °C for 48 h as previously described [25]. Briefly, 200 μl of 0.5 MacFarland turbid overnight broth culture was added to 96-well flat-bottom polystyrene plates. Plates were incubated for 48 h at 37 °C without shaking. Following the incubation, the bacterial cell suspension was removed and each well was gently washed with sterilized phosphate-buffered saline (pH 7.2). The plates were allowed to air-dried and 100 μL of 0.1% crystal violet (CV) was added to each well, allowed for 20 min and then washed three times to remove the stain. To each well, 100 μL of 85% ethanol was added to dissolve the Crystal violet. Its absorbance was measured at optical density (OD570) using a UV-microplate reader, and the level of biofilm formation was evaluated.

2.7. Phenotypic detection of virulence factors

Activities of produced hemolysin, lipase and protease by the strains were phenotypically evaluated. Hemolysin production was demonstrated as described by Edberg et al. [26]. An overnight pure single colony was sub-cultured on 5% defibrinated sheep blood agar overlayed on the Nutrient agar base (Oxoid, UK) and incubated at 37 °C for 72 h. A clear halo zone indicating lysis of the red blood cells around the colony indicates haemolysin production [27]. Phenotypic assay for protease production was performed according to Suganthi et al. [28]. Briefly, pure broth of 0.5MacFarland was dropped on Skim milk agar supplemented with 1% casein and allowed to be adsorbed and incubated at 37 °C for 24 h. Casein hydrolysis was assessed as positive with an indication of a clear zone around the inoculum spot. Lipase production was detected with inoculation of a loopful of 18 h pure bacteria colonies on tributyrin agar plate and incubated at 37 °C for 24 h. The zone of hydrolysis produced by the strain was observed as an indication for lipase production [29].

2.8. Antibiotic resistance relatedness

Antibiotic resistance relatedness of *E. coli* O157 strains from human, water and animal sources were evaluated DendroUPGMA construction utility program. Binary data coded for susceptibility (1) and resistance (0) from the set of antibiotic susceptibility variables analysed for similarity using the unweighted pair group method with arithmetic averages (UPGMA) to construct a phylo-dendrogram [30,31]. Associated virulence factors (hemolysis, lipase, protease), biofilm and MARI were analysed with the dendrogram.

2.9. Geo-spatial mapping

Geographical coordinates of residence of individual subjects, point of sales of the animal products and locations of water samples with detected *E. coli* O157 strains were recorded with a differential global positioning system (GPS) and interpolated for analysis in ArcGIS programme with respect to land division according to boundary marks in southwest Nigeria [32]. Each strain was assigned to geographical coordinates based on resistant rates to antibiotics mapped with strains from various sources (human, food animal and water). The spatial heat maps were generated according to colour intensity in proportion to the resistance scale displayed in varying degrees (yellow to indicate high resistance, red is intermediate, light purple as low resistance and blue as very low-level susceptibility). To further study the spread and prevalence of the strains, bubble maps were created to show the proportion of resistance rate of each strain from different sources with respect to their geographical coordinates (Supplementary 1 Table).

2.10. Data analysis

The significance of *stx*-encoded strains prevalence was compared among the human subjects, water and food animal products using a chi-square test with Yates' correction, taking the $p < 0.05$. Odd ratios and respective associated 95% CIs were estimated for the significant association of *stx1*, *stx2*, *stx1/stx2*, conjugants, and transformants. Comparative levels of antibiotic susceptibility patterns based on MIC_{50} and MIC_{90} rates among the strains were evaluated with ANOVA. The abundance and proportion of antibiotic susceptible and resistant strains from different sources were compared using heatmap generated with Graph Padprism6. The association and possible relatedness of human, water and food animal *Escherichia coli* O157 strains was assessed with Principal component analysis (PCA) based on the factors for antibiotic resistance and the calculated number of principal components with eigenvalues >1.00 [33].

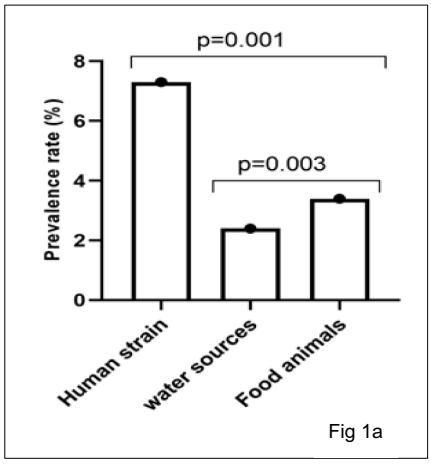
3. Results

3.1. Prevalence rate and stx-carriage level

The prevalence rates of *E. coli* O157 Latex-detected human strains (7.56%) were significantly higher compared to water and food animals ($p = 0.001$) (Fig. 1a). Higher proportion of *stx1/stx2* (53/66) (OR(95% CI) = 34.65(94.5), $p = 0.023$) obtained from human disease conditions (diarrhea, UTI, colitis, Skin and soft tissue, HUS) were recorded compare to strains from food animal (OR(95%CI) = 73.82(94.5), $p = 0.037$) and water sources (OR(95%CI) = 34.65(94.5), $p = 0.563$, Fig. 1b).

4. Resistance at Sub-MIC

Median resistance to ciprofloxacin, gentamycin, ofloxacin and nitrofurantoin (Fig. 2A, B, D, H) shown by water *Escherichia coli* O157 were significantly higher compared to low-level resistance recorded among the human and food animals strains ($p < 0.05$). <20% median resistance to gentamycin, $p = 0.042$ (Fig. 2B) and doxycycline (Fig. 2E) was recorded ($p = 0.063$). Considering the minimum inhibitory concentration levels of *Escherichia coli* O157 (Table 1), highest resistance of 84.5%, 81.3%, 70.7% and 62.1% to doxycycline (MIC_{50} 8 μg/mL and MIC_{90} 128 μg/mL; $p = 0.018$), tetracycline (MIC_{50} 4 μg/mL and MIC_{90} 64 μg/mL; $p = 0.001$), ciprofloxacin (MIC_{50} 2 μg/mL and MIC_{90} 128 μg/mL; $p = 0.001$) and gentamycin (MIC_{50} 4 μg/mL and MIC_{90} 256 μg/mL; $p = 0.002$) were respectively recorded among human subjects and significantly higher compared to lower resistance rates among strains



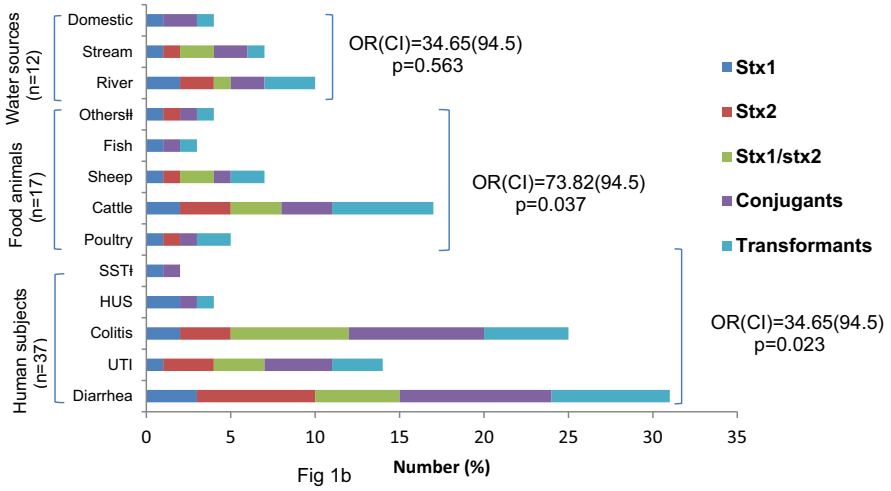


Fig 1. a. Prevalence rates of latex-positive *Escherichia coli* O157 strains obtained from human subjects, water sources and food animals. Fig 1b; Evaluation of shiga toxin genes (stx1, stx2, stx1/stx2), hfr-conjugants and transformants prevalent in different sources (Key: ‡SST, Skin and Sub-cutaneous tissues; ‡‡Others, including ruminant livestock; ‡‡Domestic waters, including household water storage containers, overhead water tank).

from food animals and water sources.

4.1. Resistance relatedness

Comparative resistance relatedness with expressed virulent factors reveals clustering of *Escherichia coli* O157 from human diarrhea ($n = 13$), SST (n = 1), UTI (n = 13), colitis ($n = 4$), HUS (n = 1), cattle ($n = 5$), fish (n = 4), sheep (n = 4), poultry ($n = 3$), well (n = 1), stream ($n = 2$), and river (n = 5) with resistance to four to seven antibiotics, R-plasmid (14.03 ± 1.24kbp), MARI (0.75), and high production of biofilm, hemolysin and low-level lipase and protease expression grouped into phylo-group F (Fig. 3). Different phylo-clusters characterized with biofilm and hemolytic productions were observed in group A with resistance to four and eight antibiotics, average R-plasmid 14.39kbp and MARI (0.84) including strains from human diarrhea, SST, UTI, colitis, cattle, fish, sheep, groundwater (wells), streams, and rivers.

4.2. Cluster analysis

A high heterogeneous resistance pattern observed among strains from diarrheic (15/16), colitis (2/4) and UTI (11/14) were resistant to amoxicillin, tetracycline and doxycycline. Cattle (2/16) and poultry (3/16) showed high resistance rates to tetracycline and amoxicillin while very low susceptibility to gentamycin in cattle was recorded. <12% of water strains particularly from rivers comprised most dominant resistance to ceftazidime, tetracycline and gentamycin (Fig. 4a). Principal component analysis revealed the association between antibiotic resistance and abundance of *Escherichia coli* O157 obtained from human sources with strains from food animal products and various water sources. In Fig. 4b, the score plot demonstrated a differential proportion of human, food animal and water strains with related antibiotic resistance (cluster 3) and high-level resistance in cluster 2 (including strains from human diarrhea and UTI, poultry, cattle, fish, rivers and streams). Large variance was observed in clusters 1 and 4 with low susceptibility to gentamycin, cefuroxime, ciprofloxacin, and chloramphenicol. The observed variance in low susceptible strains only includes human and water strains. Similar low variance in antibiotic resistance among the human and food animal strains further suggest a related resistance pattern (Fig. 4c). A high proportion of human strains clustering with water strains (cluster a) was important indices of low-level association similarly recorded in cluster b (Fig. 4d).

4.3. Geospatial mapping

Spatial spread of the resistant food animal *Escherichia coli* O157 reveals high-level resistance *stx1* and *stx2* encoded strains disseminated within and around the urban metropolis. A low susceptibility was observed in less populated locations not far from major cities (Fig. 5A). Focal and dense populations of heterogeneous *stx1, stx2* and *stx1/2* encoded strains from subjects with diarrhea and colitis were majorly found in urban locations. Though, sparsely distributed strains in urban, sub-urban and rural areas showed a very low susceptibility rate (Fig. 5B). Major streams and rivers in urban settlements located within and around major cities harbor mostly high resistance strains encoded with *stx1* and *stx2* genes but a low susceptible strain was predominantly found in sub-urban and rural locations (Fig. 5C). Holistic surveillance for the *Escherichia coli* O157 strains and their abundance was explored (Fig. 5D) showing the abundance of food animals, and water *Escherichia coli* O157 strains spreading with human strains, particularly in major cities. Human strains were sparsely distributed in many suburbs and rural communities where animal and water strains were very scanty.

5. Discussion

Escherichia coli O157 infection is a global challenge arising from the consumption of contaminated food animal products, particularly beef, meat, buffon and water used for domestic purposes [34]. The recorded prevalence rate of *E. coli* O157 further substantiates the colonization of the intestinal tract due to consumption of contaminated drinking water and various animal products [35]. A significant proportion of conjugative strains encoded with *stx1* and *stx2* genes had the potential to transform normal microbiota to pathogenic strains leading to hemorrhagic colitis and HUS [36]. Continuous systemic or organ failure could be sustained as shiga toxin production persists in *Escherichia coli* O157-diarrheic conditions [14] while the association with other extra-intestinal infections maintains constant prevalence. Poorly prepared ready-to-eat meat products, untreated domestic water and an unhygienic food environment are potential reservoirs for *Escherichia coli* O157 strains, facilitating undetected transmission within and around several communities.

Observation of community-wide antibiotic resistant *Escherichia coli* O157 in food animal products and water sources are possible causes for devastating public health and severe intestinal morbidity. High antibiotic resistance (AR) of water- and animal- *Escherichia coli* O157 to ciprofloxacin, gentamycin and ofloxacin, which are first-line drugs

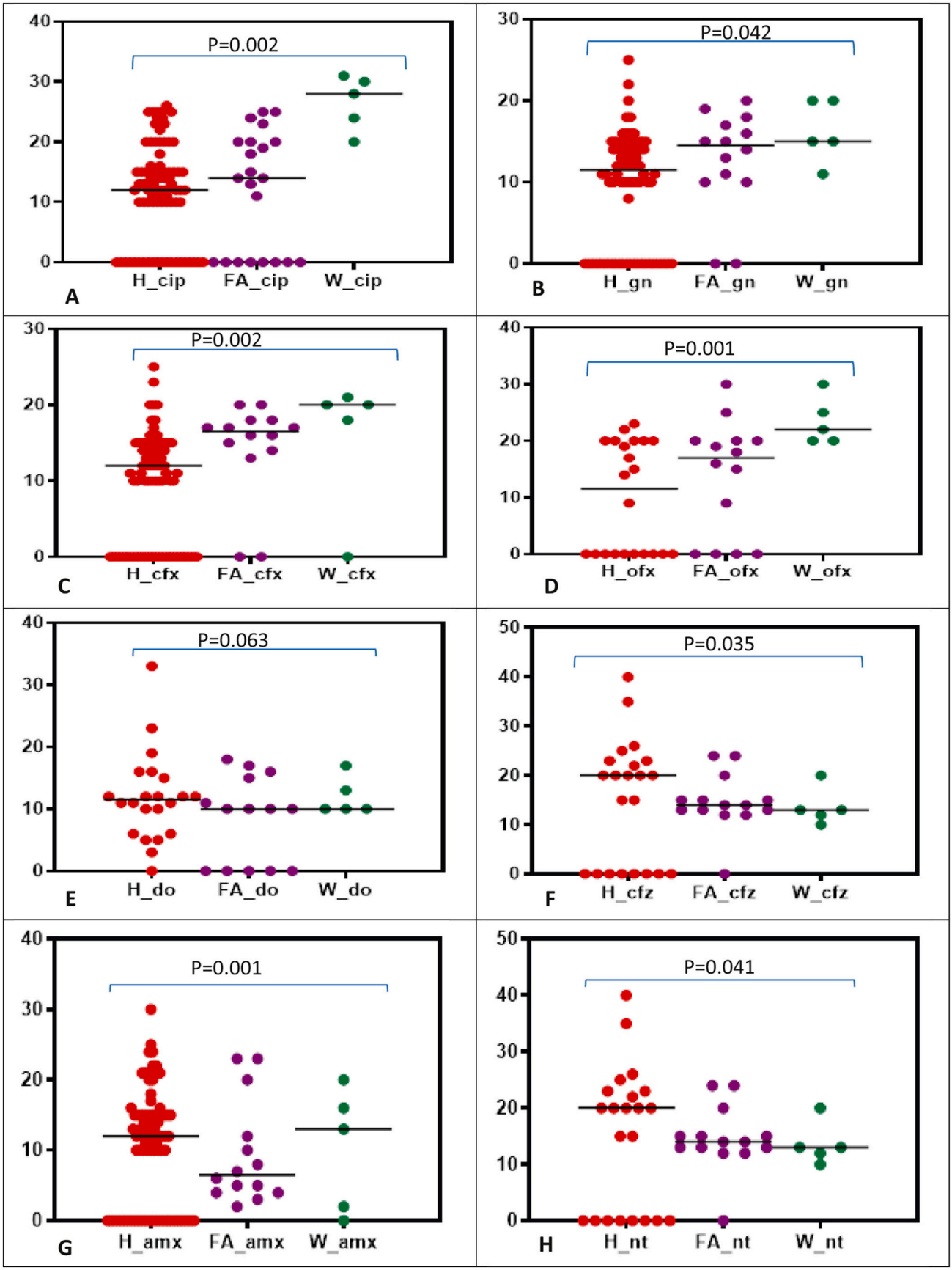


Fig 2. Scatter plot showing phenotypic antibiotic resistance pattern of detected stx-strains with horizontal line showing the median values [H, human strains (red); FA, Food animal (purple) and W, water strains (green); cip, ciprofloxacin, gn, gentamycin; cfx, cefuroxime; ofx, ofloxacin; do, doxyclicne; cfz, ceftazidime; amx, amoxicillin; nt, nitrofurantoin].

Table 1
Antibiotics minimum inhibitory concentrations against *Escherichia coli* O157 strains obtained from various sources.

Antibiotics	BP (μg/mL)	Human (*N* = 37)			Water (N = 12)			Animal (*N* = 16)			*P* value
		%R	MIC_{50}	MIC_{90}	*%R*	MIC_{50}	MIC_{90}	*%R*	MIC_{50}	MIC_{90}	
Augmentin	≥32	27.8	4	128	20.4	4	128	31.2	4	256	0.002
Nitrofunrantoin	≥16	37.9	4	256	54.2	4	128	71.4	4	128	0.001
Ciprofloxacin	≥8	70.7	2	128	34.0	1	64	57.1	8	256	0.001
Ceftazidime	≥32	50.0	8	256	25.9	8	64	28.6	8	128	0.001
Cefuroxime	≥32	53.5	8	256	38.3	8	64	35.7	16	128	0.012
Gentamycin	≥16	62.1	4	256	58.5	2	64	64.3	4	256	0.002
Doxycycline	≥32	84.5	8	128	74.8	4	128	68.6	8	128	0.018
Ofloxacin	≥8	39.7	2	64	46.5	2	64	42.9	4	256	0.001
Amoxycillin	≥32	36.2	8	128	38.2	8	256	64.3	8	128	0.001
Tetracycline	≥ 16	81.3	4	64	41.0	8	256	50.0	8	256	0.001

(BP; CLSI breakpoint of resistant strains determined at BP equal or above the BP; %R percentage of resistant strains.)

provide evidence of possible resistance gene transmission from fecal shedding or feed into rivers, streams and wells [37]. Consequence of the strain resistance to doxycycline (MIC_{50} 8 μg/mL and MIC_{90} 128 μg/mL), tetracycline (MIC_{50} 4 μg/mL and MIC_{90} 64 μg/mL), ciprofloxacin (MIC_{50} 2 μg/mL and MIC_{90} 128 μg/mL) and gentamycin (MIC_{50} 4 μg/mL and MIC_{90} 256 μg/mL) present multi-drug resistance spectrum suggesting possible high treatment failure, multiple colon pathology and outbreak of MDR- *Escherichia coli* O157.

Resistance to doxycycline and tetracycline was similar to implicated *tet* determinants reported in diarrheic patients with intestinal inflammation and colitis [35,36], gentamycin resistance in severe UTI [38], diagnosed HUS [4] and soft tissue abscesses [15]. Tetracycline and gentamycin resistance in pigs, cattle and chicken were associated with human diarrheic strains and expressed high MIC levels [39]. Evidence of resistance patterns of the human pathotypes with animal strains could enhance increased treatment failure and severe intestinal *Escherichia coli* O157 infection. Activation of enzymes, acquisition of horizontal transfer potential via conjugative or transformative genetic elements, activation of efflux pumps [40] and mutational alteration of target sites [41], are possible mechanisms for active resistance dissemination.

To tackle the prevalence and transmission rate of *Escherichia coli* O157, One Health approach that includes relatedness of virulence factor production and antibiotic resistance of the human, water and animal strains are critical factors to be considered. Comparative analysis of AR among strains from human diarrheic, UTI, colitis with high MARI, putative R-plasmid carriage, biofilm and hemolysin production presents continuous dissemination and active drive for resistance strain distribution. Relatedness of the hemolysin production implies functional tissue damage, dysfunctional cellular immune responses and destructive target for red cells leading to severe hemolysis [42,43]. Acquisition of hemolysin genes from animal or water strains could induce enterohemorrahgic conditions associated with bloody stool and urine in complicated *Escherichia coli* O157 infection [4]. Protease production among the isolated *stx*-variants increases the virulence capacity owing to increased extracellular protease expression that facilitates tissue invasion, subversion of host defenses, and enhancement of other virulence factors [34]. The complexity of protease-*stx* interaction in *Escherichia coli* O157 and other regulatory determinants need to be assessed for critical evaluation of the clinical impact of diverse *stx*-variants among the populace [28].

Differential phylo-groups of human *Escherichia coli* O157 (from diarrheagenic and UTI conditions) with high biofilm production related to strains from cattle, fish, streams and rivers, suggest effective adherence capability to the epithelia, urothelium cells and other cellular surfaces for colonization [5,44]. Production of biofilm would further enhance protection from antimicrobial agents and intensify virulence activity and pathogenicity. The high proportion of human diarrheic, colitis, UTI strains showing resistance to tetracycline, amoxicillin, doxycycline, gentamycin, and ceftazidime clustering with cattle and poultry further explains the frequent genetic exchange of resistance determinants [27]. This zoonotic resistance pattern and transmission could further increase antimicrobial resistance spread, treatment failure and intestinal morbidity through the consumption of antibiotic residue in meat [12]. The observed increasing antibiotic resistance in this locality is not limited to clinical settings but it has been reported in poultry [45], fish [46] and ruminant farming [47], and river waters [8], while environmental resistome from antibiotic residue in surface and underground water sources will also increase [48]. Behavioral interaction and frequent misuse of antibiotics in poultry for prophylaxis, ruminant feed additives intensify AR dissemination and enhance water bodies to serve as reservoirs for different antibiotic residues [48]. Clustering and genetic interaction of encoded AR genes in human and animal strains give insight into the re-emergence of multi-drug resistance (MDR) that could reduce the functional activity of commonly used antibiotics for the treatment of severe diarrheic infection, colitis and UTI [4].

Reports of MDR-*Escherichia coli* O157 in rivers and domesticated public streams that receive municipal sewage and other waste portray public health hazards that could enhance increasing MDR population and spread [49]. Presentation of PCA multivariate analysis and differential proportion of human, food animal and water strains with related antibiotic resistance detected among clusters justify the association of *Escherichia coli* O157 from human sources with poultry, cattle, fish, rivers, and streams. Recorded high-level AR variance among the strains from different microbial communities provides information on the future adverse impact on the well-being and socio-economic output, which need further investigations. Other AR variables in human strains illustrate significant relatedness with cattle and poultry strains, giving insight into the active zoonotic transmission of *Escherichia coli* O157 that could re-circulate from water sources to human via drinking or domestic water [48]. Frequent seepage and voluminous outflow of sewage and abattoir waste into streams and rivers provide strains induction of AR and confer resistance to other bacteria strains in the water body, thereby enhancing human infection and serving as a potential source of food animal contamination [26,50]. The environmental impact of streams and rivers (commonly used for domestic purposes) on human health needs regular evaluation and fecal contamination monitoring. Demographic surveillance towards reducing the community spread and focal outbreak of newly emerging MDR variants is required.

The spread of *stx*-encoded *Escherichia coli* O157 strains with high-level antibiotic resistance in studied communities signifies growing MDR challenges that could limit effective treatment options and the severity of *Escherichia coli* O157 infection. Recorded spatial dissemination of low antibiotic susceptible food animal- *Escherichia coli* O157 strains within and around urban locations present the communities as high–risk areas with possible wider spread due to population density. Spatial variation of *stx* and AR spread provides a subjective impression of the high population having uncontrolled access to antibiotics for various infections without prescription, such as feed additives,

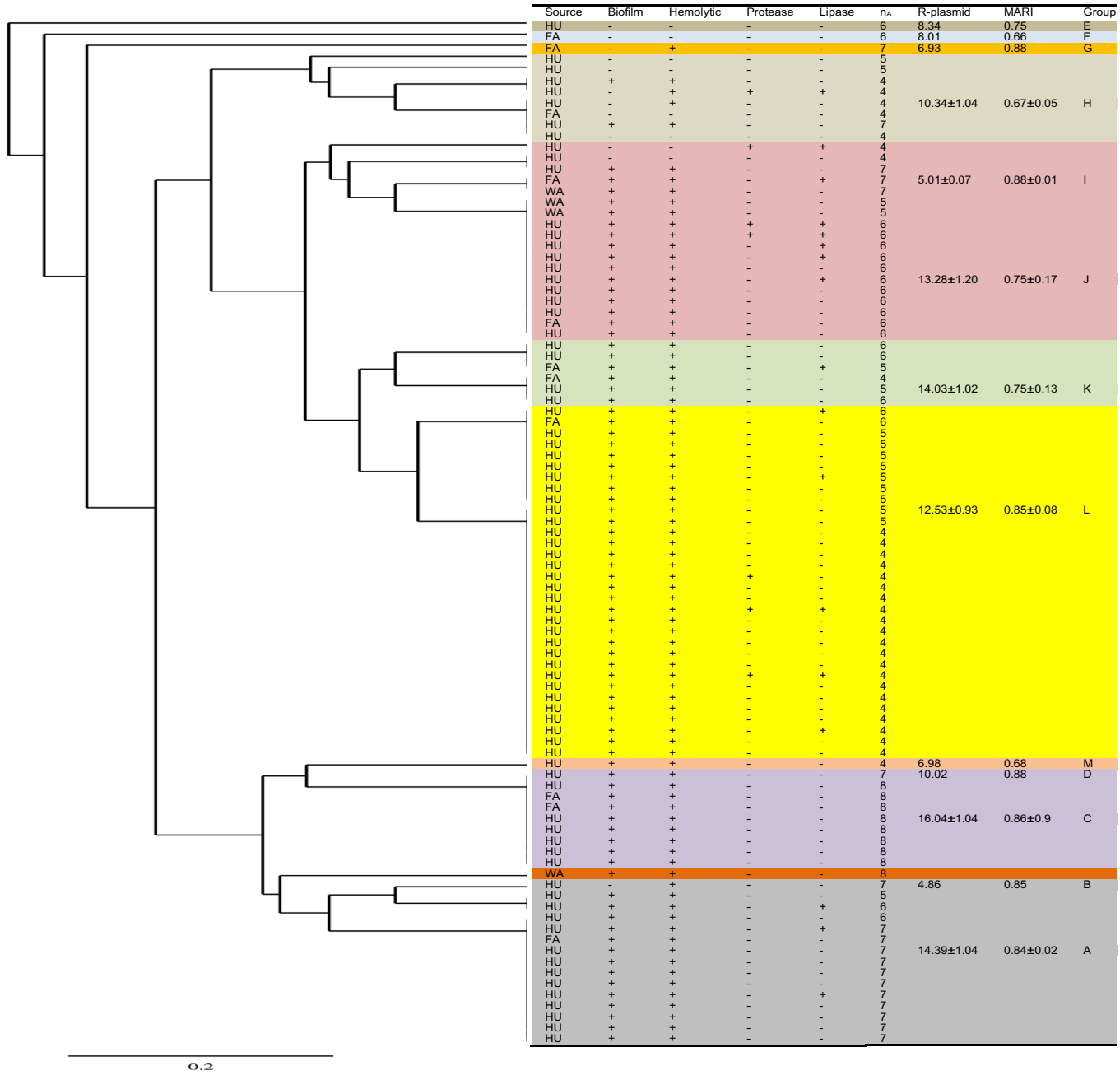
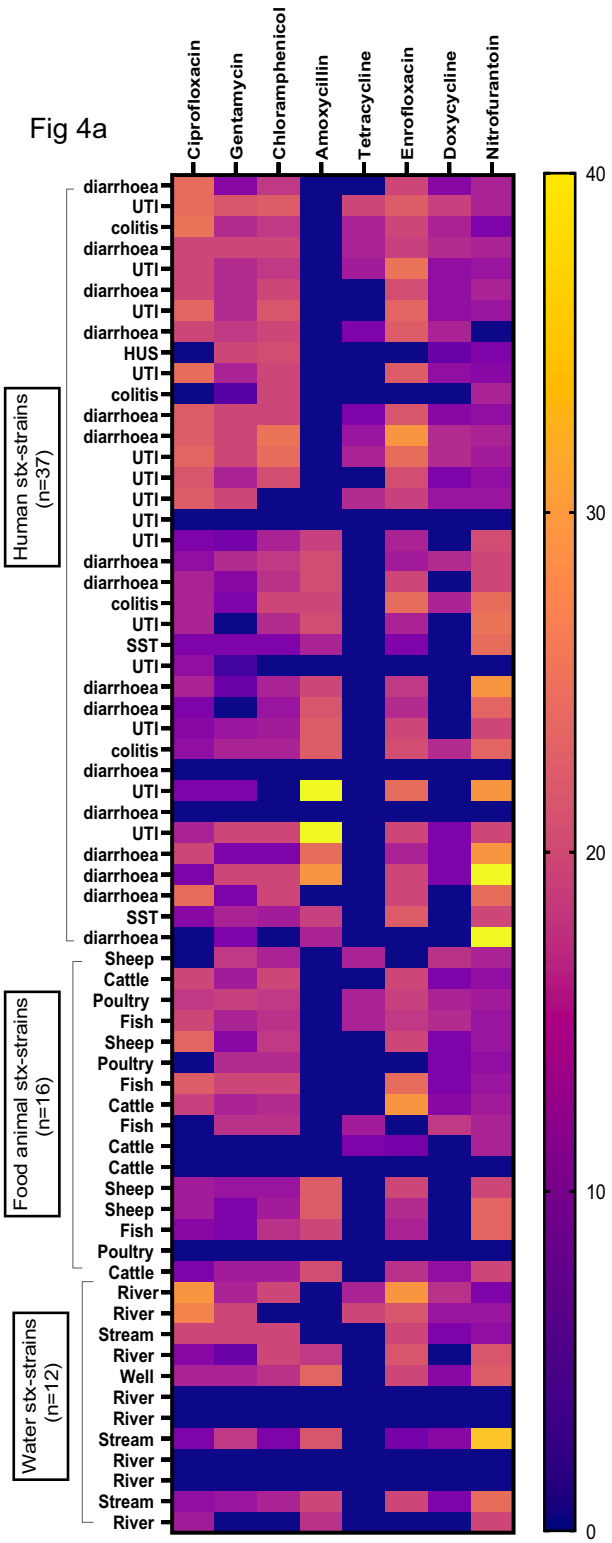


Fig 3. Analysis of virulent factor production, encoded R-plasmid and MARI relatedness of the EC O157 strains obtained from various sources (n_A, number of antibiotic resisted; G, phylo-group).

household-water treatment agents and animal husbandry. Poor public health infrastructures in many urban and rural communities are possible factors for the occasional occurrence and spread of *Escherichia coli* O157 infection with a high propensity [51].

The heterogeneous distribution of low susceptible *stx*-variants among the subjects with diarrhea and colitis in major urban locations further suggests a continuum focal spread in densely populated urban areas. The negligence of national and local health authorities to *Escherichia coli* O157 infection in some geographical locations with high prevalence intensifies community-acquire *Escherichia coli* O157 infection rates that could give rise to high intestinal morbidity. Devastating ecological factors, environment, declining population, demographic status and unhygienic consumption of animal products are possible associated risk factors driving the incidence and prevalence of *stx*-resistant strains in several communities. Strategic use of spatial data to identify areas with significant MDR encoded *stx*-variants, particularly the strains implicated in intestinal infections, shall provide insight into the possible risk of infection, dynamics of rural and urban transmission, and application of different preventive approaches [52,53]. Mapping major streams and rivers in urban settlements for MDR strains encoded with *stx1* and *stx2* genes is required to assess water contamination, bacteria-antibacterial drug interaction, and practical empiric intervention for prevention. Seepage of sewage, constant flow of abattoir wastewater and defecation into water bodies are noticeable practices in rural and urban communities, thereby facilitating MDR burden, enhancing the magnitude of *stx* carriage and observed geographical trends [54].

The spatial pattern of *Escherichia coli* O157 abundance in food



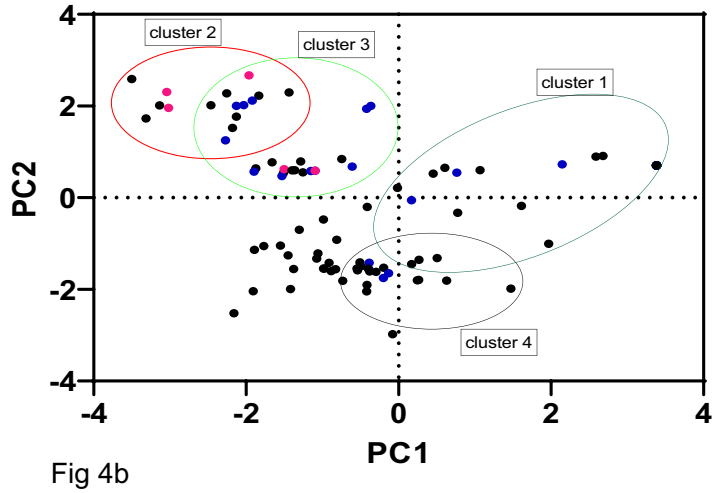


Fig 4b

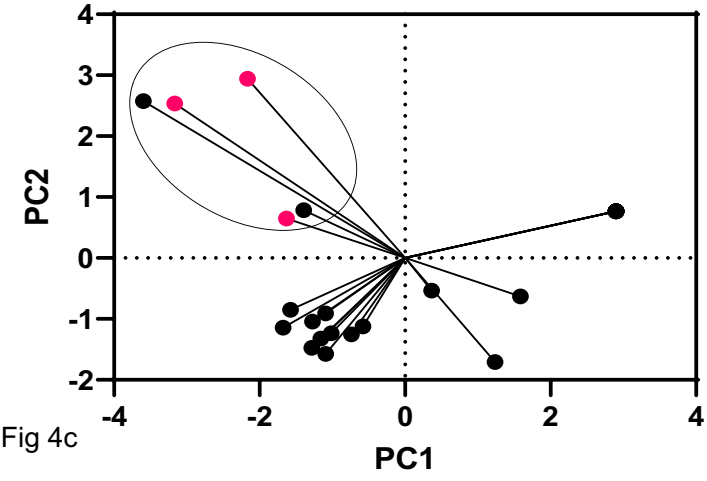


Fig 4c

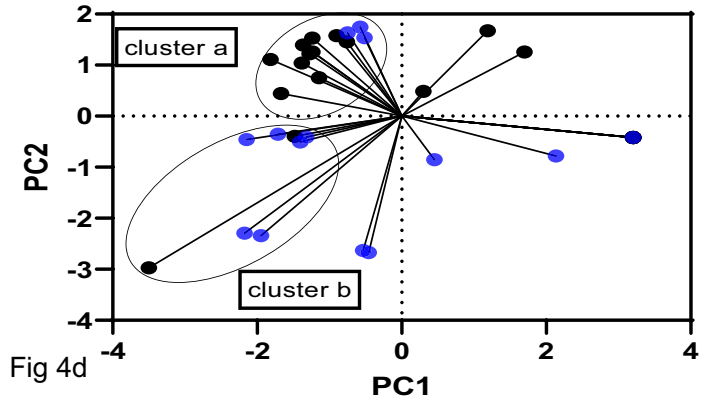


Fig 4d

Fig. 4. (a).Heatmap of the antibiotic-resistant profile of stx-strains from various human disease conditions, types of food animals and sources of water with commonly used antibiotics. Fig. 4 (b) Principal Component Analysis (PCA) plots showing antibiotic resistance profiles of human strains (black) with food animal (brick red) and water strains (blue) in ellipse clustering at 95% confidence interval (c) Separate related resistant profile analysis of human (black) and water strains (pink) on first and second principal component (d) PCA biplots of antibiotic resistance profiles of human (black) and food animal (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

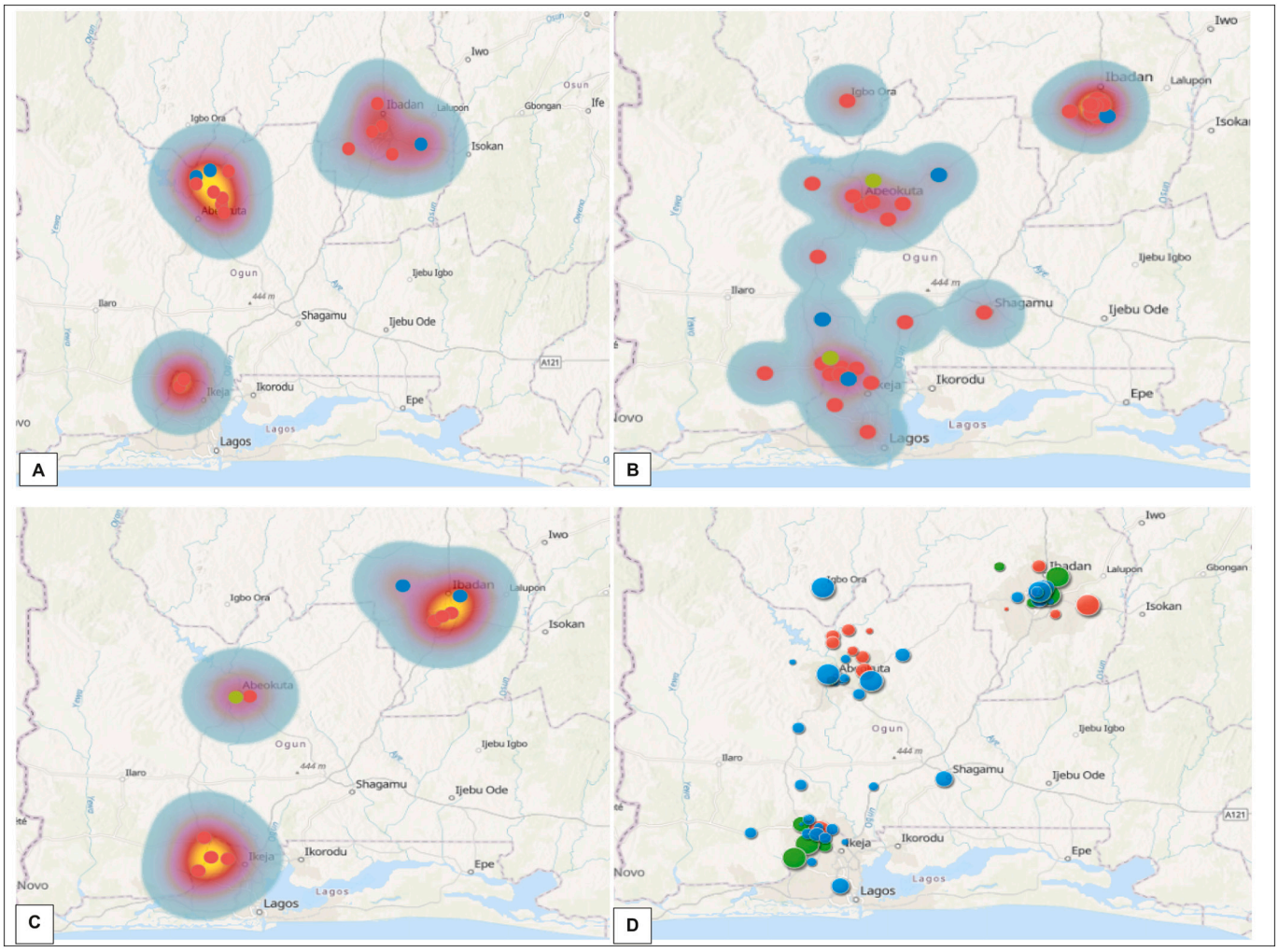


Fig. 5. Spatial distribution and proportion of *Escherichia coli* O157 antibiotic resistance hotspots in southwest Nigeria (Red, Human strains; Green. Water strains; Blue, Animal strains).

animals posed challenges of tracking the source of abattoirs where the animals were slaughtered and possible contamination of fecal shedding. The spatial spread of food animal and human strains in major cities provides evidence of the association between human and disease ecology of ruminant animals, particularly cattle and sheep. Human behavioral activities with *Escherichia coli* O157 asymptomatic cattle, poultry and sheep and possible consumption of unpasteurized milk, meat, and fecal contamination of food animal products, aid spatial transmission predisposing humans to risk of infection.

The study could not explain the factors responsible for the low prevalence and sparse distribution of human *stx*-strains in many suburbs and rural communities where high animal husbandry is the practice [55]. The zoonotic spread could be intensified from contact with animal manure, local and poor agricultural implements for farming practices. Insufficient production and processing chain of food animal products for public consumption expose the community to imminent *Escherichia coli* O157 outbreak. Spatial comparison and mapping of *stx*-variant with GIS is highly helpful in organizing the available data and communicating the results towards generating appropriate hypotheses on disease causation, spread, and prevention [56].

6. Conclusion

Continuous neglect of *stx*-encoded *Escherichia coli* O157 with a high conjugative ability to propel spread remains a worrisome concern to community well-being. High antibiotic relatedness of human diarrheagenic strains with water and animal strains from various communities poses a potential risk to public health. Therefore, strategic interventions through the One Health approach are needed to mitigate the increasing intestinal morbidity and practical integration of population-target antimicrobial stewardship to immensely reduce mortality impact and resistant cluster dissemination. Emphasis on the regular application of spatial data on antimicrobial resistance spread is a key to antimicrobial surveillance and the provision of achievable framework for monitoring, validation, and surveillance of antimicrobial resistance transmission of zoonotic food-borne *Escherichia coli* O157 pathogens.

6.1. Study limitations

Detailed demographic data of the participating subjects could not be fully retrieved, and the sources of food animal products were difficult to track. Recorded geographical coordinates at the point of sale of the food animal products were stored but the sources of these animals could not be tracked.

Data availability

All relevant data are within the manuscript and its Supporting Information files.

Financial disclosure

None.

Transparency declarations

None to declare.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2022.100447.

References

[1] A.M. Smith, N.P. Tau, B.J. Kalule, M.P. Nicol, M. McCulloch, C.A. Jacobs, K. M. McCarthy, A. Ismail, M. Allam, J. Kleynhans, Shiga toxin-producing Escherichia coli O26: H11 associated with a cluster of haemolytic uraemic syndrome cases in South Africa, Access Microbiology 1 (9) (2017).
[2] M.A. Ameer, A. Wasey, P. Salen, Escherichia Coli (E Coli 0157 H7), StatPearls [Internet], 2020. May 28.
[3] E.M. Cody, B.P. Dixon, Hemolytic uremic syndrome, Pediatr. Clin. 66 (1) (2019 Feb 1) 235–246.
[4] H. Andersen, Children on the frontline against E. coli typical hemolytic-uremic syndrome, J. Clin. Lab. Sci. 18 (2015) 90–99.
[5] O.A. Olowe, B.W. Aboderin, O.O. Idris, et al., Genotypes and phenotypes of Shiga toxin-producing Escherichia coli (STEC) in Abeokuta, Southwestern Nigeria, Infect. Drug Resist. 7 (2014) 253–259, https://doi.org/10.2147/IDR.S66268.
[6] J.Y. Magaji, C.D. Chup, The effects of abattoir waste on water quality in Gwagwalada-Abuja, Nigeria, Ethiop. J. Environ. Stud. Manag. EJESM. 5 (4) (2012) (Suppl.2).
[7] Chase E. Golden, Michael J. Rothrock, Abhinav Mishra, Mapping foodborne pathogen contamination throughout the conventional and alternative poultry supply chains, Poult. Sci. 100 (7) (2021).
[8] S. Sharma, A. Bhattacharya, Drinking water contamination and treatment techniques, Appl Water Sci 7 (2017) 1043–1067, https://doi.org/10.1007/s13201-016-0455-7.
[9] Athumani M. Lupindu, Epidemiology of Shiga toxin-producing Escherichiacoli O157:H7 in Africa in review, Southern African, J. Infect. Dis. 1 (33) (2018) 24–30, https://doi.org/10.1080/23120053.2017.1376558.
[10] H. Howie, A. Mukerjee, J. Cowden, J. Leith, T. Reid, Investigation of an outbreak of Escherichia coli O157 infection caused by environmental exposure at a scout camp, Epidemiol. Infect. 131 (3) (2003) 1063–1069, https://doi.org/10.1017/S0950268803001250.
[11] Collins Njie Ateba and Boitumelo Itiel Marumo, Isolation of enterohaemorrhagic *Escherichia coli* O104 strains from raw meat products in the north West Province, South Africa, J. Food Nutrit. Res. 2 (6) (2014) 288–293, https://doi.org/10.12691/jfnr-2-6-4.
[12] O.A. Ayodele, A.M. Deji-Agboola, P.A. Akinduti, A.O. Faneye, Phylo-diversity of prevalent human E. coli O157: H7 with strains from retailed meat and fish in selected markets in Ibadan Nigeria, J. Immunoass. Immunochem. 41 (2) (2020 Mar 3) 117–131.
[13] A.M. Deji-Agboola, N.O. Sunmola, J.A. Osiyemi, S.O. Makanjuola, P.A. Akinduti, O. Ejilude, E.O. Osiyemi, Incidence of enterotoxigenic *Escherichia coli* in Slaughter Houses in Sagamu, Nigeria, J. Adv. Microbiol. 9 (3) (2018) 1–8.
[14] O. Shittu, A. Oladotun, G. Oluwasanya, P.A. Akinduti, A. Akintokun, Occurrence of multi-antibiotic resistant Escherichia coli O157:H7 in commercial packaged sachet drinking water in Abeokuta, Nigeria, J. Adv. Microbiol. 12 (4) (2018) 1–11.
[15] K.K. Addo, G.I. Mensah, K.G. Aning, N. Nartey, G.K. Nipah, C. Bonsu, M.L. Akyeh, H.L. Smits, Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana, Tropical Med. Int. Health 16 (2) (2011 Feb) 227–232, https://doi.org/10.1111/j.1365-3156.2010.02666.x. Epub 2010 Nov 10. PMID: 21070512.
[16] A. Walusansa, J.S. Iramiot, C.F. Najjuka, D. Aruhomukama, H.K. Mukasa, H. Kajumbula, B.B. Asiimwe, High prevalence of antibiotic resistant Escherichia coli serotype O157: H7 among pastoral communities in rural Uganda, Microbiol. Res. J. Int. 30 (6) (2020) 36–43, https://doi.org/10.9734/mrji/2020/v30i630230.
[17] S.E. Majowicz, E. Scallan, A. Jones-Bitton, J.M. Sargeant, J. Stapleton, F.J. Angulo, D.H. Yeung, M.D. Kirk, Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis, Foodborne Pathog. Dis. 11 (6) (2014 Jun) 447–455, https://doi.org/10.1089/fpd.2013.1704.
[18] J.P. Nataro, J.B. Kaper, Diarrheagenic Escherichia coli, Clin. Microbiol. Rev. 11 (1) (1998 Jan) 142–201, https://doi.org/10.1128/CMR.11.1.142. Erratum in: Clin Microbiol Rev 1998 Apr;11(2):403. PMID: 9457432; PMCID: PMC121379.
[19] P.A. Akinduti, B.W. Aboderin, Rasaq Oloyede, Joseph I. Ogiogwa, Babatunde O. Motayo, Oluwaseun Ejilude, High-level multi-resistant and virulent Escherichia coli in Abeokuta, Nigeria, J. Immunoass. Immunochem. 37 (2) (2016) 104–114, https://doi.org/10.1080/15321819.2015.1063504.
[20] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Pathol. 45 (1966) 493–496.
[21] CLSI (Ed.), Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI document M100–S27, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, 2017.
[22] G.A. Denys, S.M. Callister, M.J. Dowzicky, Antimicrobial susceptibility among gram-negative isolates collected in the USA between 2005 and 2011 as part of the Tigecycline evaluation and surveillance trial (T.E.S.T.), Ann. Clin. Microbiol. Antimicrob. 12 (2013) 24, https://doi.org/10.1186/1476-0711-12-24.
[23] I. Feliciello, G. Chinali, A modified alkaline lysis method for the preparation of highly purified plasmid DNA from Escherichia coli, Anal. Biochem. 1;212 (2) (1993) 394–401, https://doi.org/10.1006/abio.1993.1346. PMID: 8214582.
[24] Chyi-Liang Chen, Su Lin-Hui, Rajendra Prasad Janapatla, Chun-Yen Lin, Cheng-Hsun Chiu, Genetic analysis of virulence and antimicrobial-resistant plasmid pOU7519 in Salmonella enterica serovar Choleraesuis, J. Microbiol. Immunol. Infect. 53 (1) (2020) 49–59.
[25] J. Leech, S. Golub, W. Allan, et al., Non-pathogenic Escherichia coli biofilms: effects of growth conditions and surface properties on structure and curli gene expression, Arch. Microbiol. (2020) 1517–1527, https://doi.org/10.1007/s00203-020-01864-5.
[26] S.C. Edberg, P. Gallo, C. Kontnick, Analysis of the virulence characteristics of bacteria isolated from bottled, water cooler, and tap water, Microb. Ecol. Health Dis. 9 (2) (1996) 67–77.
[27] E.S. Lopes, W.M. Cardoso, D.M. Nishi, R.V. Horn, Á.H. Albuquerque, S.V.G. Lima, A.J.F. Beleza, F.C. Gaio, C.C. Carmo, M.N. Pascoal Filho, R.S.C. Teixeira, Sero-group identification, phenotypic detection of hemolysis and extended spectrum beta-lactamases of Escherichia coli isolated from psittacine of illegal wildlife trade in Fortaleza, Brazil, Arq. Bras. Med. Vet. Zootec. 70 (3) (2018) 823–829.
[28] C. Suganthi, A. Mageswari, S. Karthikeyan, M. Anbalagan, A. Sivakumar, K. M. Gothandam, Screening and optimization of protease production from a halotolerant *Bacillus licheniformis* isolated from saltern sediments, J. Genet. Eng. Biotechnol. 11 (1) (2013) 47–52.
[29] E. Mobarak-Qamsari, R. Kasra-Kermanshahi, Z. Moosavi-Nejad, Isolation and identification of a novel, lipase-producing bacterium, pseudomonas aeruginosa KM110, Iran. J. Microbiol. 3 (2) (2011) 92–98.
[30] S. Garcia-Vallvé, J. Palau, A. Romeu, Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in Escherichia coli and Bacillus subtilis, Mol. Biol. Evol. 16 (1999) 1125–1134.
[31] P.A. Akinduti, A. Oluwadun, J. Osiyemi, O. Ejilude, P.O. Isibor, Multi-antibiotics Resistant Relatedness of bla-gene Encoded Enteric bacteria harbouring High Molecular R-plasmids. IOP Conf. Ser.: Earth Environ. Sci, 2018, 210 012002.
[32] S. Galvin, N. Bergin, R. Hennessy, B. Hanahoe, A.W. Murphy, M. Cormican, A. Vellinga, Exploratory spatial mapping of the occurrence of antimicrobial resistance in E. coli in the community, Antibiotics. 2 (3) (2013) 328–338, https://doi.org/10.3390/antibiotics2030328.
[33] N. Russo, A. Stamilla, G. Cascone, C.L. Randazzo, A. Messina, M. Lanza, A. Pino, C. Caggia, F. Antoci, The wide range of antibiotic resistance and variability of genotypic profiles in Escherichia coli from domestic animals in eastern Sicily, Antibiotics 10 (2021) 28, https://doi.org/10.3390/antibiotics10010028.
[34] Kim Jun-Seob, Lee Moo-Seung, Kim Ji Hyung, Recent updates on outbreaks of Shiga toxin-producing Escherichia coli and its potential reservoirs, Front. Cell. Infect. Microbiol. 10 (2020) 273.
[35] W.K. Sang, V. Oundo, D. Schnabel, Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhoea in four provinces of Kenya, J. Infect. Dev. Ctries. 6 (7) (2012 Jul 23) 572–578, https://doi.org/10.3855/jidc.2196 (PMID: 22842944).
[36] Maryam Javadi, Saeid Bouzari, Mana Oloomi, Horizontal gene transfer and the diversity of *Escherichia coli*. *Escherichia coli*, in: Recent Advances on Physiology, Pathogenesis and Biotechnological Applications, 2017, pp. 317–331.
[37] S. Olorunleke, *Chemical Composition, Bacterial Load and Prevalance of Verotoxigenic Escherichia Coli in Donkey Meat Sold at Ezzamgbo, Ebonyi State Nigeria* (Doctoral dissertation) 2017, Ebonyi State University, 2017.
[38] Shahla Shahbazi, et al., Distribution of extended-spectrum β-lactam, quinolone and carbapenem resistance genes, and genetic diversity among uropathogenic Escherichia coli isolates in Tehran, Iran, J. Glob. Antimicrob. Resist. (14) (2018) 118–125.
[39] Nurul Asyiqin Haulisah, et al., High levels of antibiotic resistance in isolates from diseased livestock, Front. Veterin. Sci. 8 (2021) 300.

[40] Yue Wang, et al., Non-antibiotic pharmaceuticals promote the transmission of multidrug resistance plasmids through intra-and intergenera conjugation, ISME J. (2021) 1–16.
[41] Julia Maria Kloos, Horizontal transfer, selection and maintenance of antibiotic resistance determinants, 2021.
[42] Alexandra Y. Soare, et al., P2RX7 at the host-pathogen interface of infectious diseases, Microbiol. Mol. Biol. Rev. 85 (1) (2021) e00055–20.
[43] Johanna Detzner, Gottfried Pohlentz, Johannes Müthing, Valid presumption of Shiga toxin-mediated damage of developing erythrocytes in EHEC-associated hemolytic uremic syndrome, Toxins 12 (6) (2020) 373.
[44] Rune M. Pedersen, et al., A method for quantification of epithelium colonization capacity by pathogenic bacteria, Front. Cell. Infect. Microbiol. 8 (2018) 16.
[45] Christian Agyare, et al., Antibiotic use in poultry production and its effects on bacterial resistance, Antimicrob. Resist. Glob. Threat. (2018) 1–20.
[46] Yewon Lee, et al., Occurrence of pathogenic Vibrio parahaemolyticus in seafood distribution channels and their antibiotic resistance profiles in S. Korea, Lett. Appl. Microbiol. 68 (2) (2019) 128–133.
[47] Thomas C.A. Hitch, et al., Deep sequence analysis reveals the ovine rumen as a reservoir of antibiotic resistance genes, Environ. Pollut. 235 (2018) 571–575.
[48] Peng Li, et al., Occurrence and fate of antibiotic residues and antibiotic resistance genes in a reservoir with ecological purification facilities for drinking water sources, Sci. Total Environ. 707 (2020), 135276.
[49] A.H. Khan, et al., Impact, disease outbreak and the eco-hazards associated with pharmaceutical residues: a critical review, Int. J. Environ. Sci. Technol. (2021) 1–12.
[50] Thomas Bintsis, Microbial pollution and food safety, AIMS Microbiol. 4 (3) (2018) 377.
[51] Hissa M. Al-Farsi, Multidrug-Resistant Enterobacterales in Oman: Molecular Epidemiology and Therapeutic Insights, Diss. Karolinska Institutet (Sweden), 2021.
[53] A.C. Mulder, et al., Spatial effects of livestock farming on human infections with Shiga toxin-producing Escherichia coli O157 in small but densely populated regions: the case of the Netherlands, GeoHealth 4 (11) (2020) e2020GH000276.
[54] D.J. Lapworth, et al., A Review of Urban Groundwater Use and Water Quality Challenges in Sub-Saharan Africa, 2017.
[55] Vinicius Silva Castro, et al., Shiga-toxin producing Escherichia coli: pathogenicity, supershedding, diagnostic methods, occurrence, and foodborne outbreaks, Compr. Rev. Food Sci. Food Saf. 16 (6) (2017) 1269–1280.
[56] Richard Elson, et al., A spatial and temporal analysis of risk factors associated with sporadic Shiga toxin-producing Escherichia coli O157 infection in England between 2009 and 2015, Epidemiol. Infect. 146 (15) (2018) 1928–1939.

Further reading

[52] J.Y. Maillard, S.F. Bloomfield, P. Courvalin, S.Y. Essack, S. Gandra, C.P. Gerba, J. R. Rubino, E.A. Scott, Reducing antibiotic prescribing and addressing the global problem of antibiotic resistance by targeted hygiene in the home and everyday life settings: a position paper, Am. J. Infect. Control (2020 Apr 18).