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Preponderance of toxigenic *Escherichia coli* in stool pathogens correlates with toxin detection in accessible drinking-water sources

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SUMMARY

Since early detection of pathogens and their virulence factors contribute to intervention and control strategies, we assessed the enteropathogens in diarrhoea disease and investigated the link between toxigenic strains of *Escherichia coli* from stool and drinking-water sources; and determined the expression of toxin genes by antibiotic-resistant *E. coli* in Lagos, Nigeria. This was compared with isolates from diarrhoeal stool and water from Wisconsin, USA. The new Luminex xTAG GPP (Gastroplex) technique and conventional real-time PCR were used to profile enteric pathogens and *E. coli* toxin gene isolates, respectively. Results showed the pathogen profile of stool and indicated a relationship between *E. coli* toxin genes in water and stool from Lagos which was absent in Wisconsin isolates. The Gastroplex technique was efficient for multiple enteric pathogens and toxin gene detection. The co-existence of antibiotic resistance with enteroinvasive *E. coli* toxin genes suggests an additional prognostic burden on patients.

Key words: Chronic diarrhoea, *Escherichia coli* toxins, multiplex analysis, stool pathogens, water sources.

INTRODUCTION

Diarrhoeal disease is a major public health problem in developing countries and is considered the foremost cause of death [1–4] accounting for about 2 million deaths worldwide annually [5–6], afflicting persons of all age groups and status [7]. The major source of the disease is the poor quality of accessible drinking water, contaminated food, poor standard of personal hygiene and lack of appropriate sanitation. It is noteworthy that the aetiological agents of diarrhoea vary with localities within an environment, socioeconomic status and cultural practices [8]. Therefore, knowledge of the aetiological agents is arguably important for adequate address of epidemiological surveillance and proper treatment. There is a tight correlation between diarrhoeal diseases and lack of adequate water worldwide [9]. In many developing countries, there is improper waste management including the handling of faecal materials. The outcome of this is the eventual leaching of such waste matters into canals and well water through the aid of flooding. There is a wide spectrum of microbes, including bacteria, viruses

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and parasites that have been implicated, either individually or synergistically, in the aetiology of the disease [10–12]. The bacterial agents of diarrhoeal disease are commonly *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Campylobacter*, *Yersinia*, *Aeromonas*, etc. [13]. Individuals afflicted with these bacteria in developing countries benefit from antibiotic therapy often via self-medication, since the antibiotics are commonly available without prescription. However, non-compliance with regard to dosing requirements is an issue due to the cost of the antibiotics [14]. There is a consequent progressive increase in antibiotic resistance in enteric pathogens in developing countries in a manner that poses a critical public health threat [15–17].

The current approach is to ascertain that the pathogen of interest is identified using culture- and non-culture-based molecular techniques with sometimes protracted turnaround times. This issue of time is further compounded by the wide diversity of bacterial and viral aetiological agents of diarrhoeal disease [18], thus complicating accurate diagnosis and necessary surveillance in a developing country. The scenario, therefore, is that of a disease burden that could be easily addressed on a long-term basis with the provision of portable clean water, better sanitation, proper hygiene and improved nutritional practices. Immediate efforts directed at addressing the disease burden itself are hampered by lack of proper diagnosis and abuse of antibiotics.

Real-time detection of multiple aetiological agents of diarrhoea from readily available specimens, such as stool, would contribute immensely to efficient clinical and public health responses to diarrhoea outbreaks, cases of chronic diarrhoea and epidemiological surveillance. An important development in the capacity to detect multiple aetiological agents is the use of such versatile methods used in the detection of toxins elaborated by bacterial agents of diarrhoea. For instance, enterotoxigenic *E. coli* produces one or more heat labile toxins (*LT1* and *LT2*); it also produces heat stabile toxins (*STa* and *STb*), which are of extreme importance as causal virulence factors of diarrhoea [19–21]. The identification and evaluation of stool pathogens in a highly populated and large area such as Lagos metropolis in Nigeria will go a long way in providing much needed information on the preponderance of a pathogen among stool pathogens and reveal the relationship between pathogenicity and various strains of the most implicated pathogen. On this basis, we decided to adopt Lagos as a typical highly urbanized city in a developing country and investigate the breadth of enteropathogen profiles in stool in a manner that reduces the turnaround time and determines the links between toxigenic strains of *E. coli* being the most frequently found bacteria of stool and accessible drinking water. The findings on *E. coli* toxin genes were compared with similarly investigated samples in Wisconsin, USA as a validation control; with the expectation that a pool of data that will be beneficial to the public health in the city will be assembled. Moreover, since the therapeutic interventions in Lagos sometimes involve indiscriminate use of antibiotics which predisposes antibiotic resistance, we decided to investigate if there is an observable pattern in the relationship between antibiotic-resistant *E. coli* and toxin elaboration; thus revealing an additional burden on the public health of the residents of such large city.

METHODS

Materials

All chemicals and reagents were purchased from Sigma-Aldrich Chemical Company (St Louis, USA) unless otherwise stated.

Study population and samples

The study population consisted of adult patients (aged 17–57 years) from seven localities (Alimonsho, Shomolu, Oshodi, Surulere, Yaba, Ikeja, Mushin) in Lagos, Nigeria, who were attending hospital with complaints about diarrhoeal disease and consented to provide stool samples. A total of 102 stool samples were obtained: 91 chronically ill diarrhoea patients and 11 controls from healthy volunteers. Further, 25 acutely ill diarrhoea patients' samples from Milwaukee and Racine in Wisconsin state, USA were collected from consenting individuals (18–55 years). Neither patients nor controls admitted to having received antibiotic treatment within 2 weeks before submission of samples. To relate findings in stool samples with accessible water in the same localities, we collected 117 water samples from seven localities in Lagos as described above from different sources: canals, wells, sachet packed water and municipal pipe-borne 'tap' water. Also, from Milwaukee and Racine in Wisconsin state, USA, 26 water samples from similar sources, i.e. local beaches, storm drains and water

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treatment plant were collected. All samples (stool and water) were aseptically collected.

Preparation of sample and culture of *Escherichia coli*

The isolation of *E. coli* involved the inoculation of fresh stool samples onto Stuart's transport medium (Oxoid, UK), maintained on ice during transportation to the laboratory for same day processing. The water samples were transported in sterile containers on ice to the laboratory and processed in the same manner as the stool samples. The samples were inoculated into peptone water (Oxoid) and incubated at 37 °C for 18 h. The samples were subsequently streaked onto Eosin Methylene Blue (EMB) agar (Oxoid) and incubated overnight at 37 °C. Indole, Methyl Red, Voges–Proskauer reaction and Simmons' citrate (IMViC) tests were performed on the colonies that showed *E. coli* growth characteristics on EMB agar. Analytical profile index (API) 20E strips (bioMérieux, France) were also used to confirm the identity of isolates as *E. coli* [22, 23].

Susceptibility studies

The *E. coli* isolates from Lagos were exposed to 16 antibiotics (ciprofloxacin 5 μg, trimethoprim/sulfamethoxazole 1·25/23·75 μg, ceftazidime 30 μg, streptomycin 10 μg, nitrofurantoin 300 μg, ofloxacin 5 μg, amoxycillin/clavulanate potassium 20 μg/10 μg, gentamicin 10 μg, cefaclor 30 μg, ampicillin 10 μg, norfloxacin 10 μg, tetracycline 30 μg, cefuroxime sodium 30 μg, nalidixic acid 30 μg, ceftriaxone 30 μg, cefixime 30 μg) reflecting different classes of antibiotics. This enabled the capture of the susceptibility pattern using disk diffusion on Mueller–Hinton agar (MHA) plates. All the *E. coli* strains showing resistance to the various antibiotics used in the susceptibility studies were further selected for virulence toxin detection using conventional multiplex real-time PCR described below.

Luminex xTag Gastrointestinal Pathogen Panel (GPP) procedure (Luminex Corp., USA)

The profiling of enteropathogens was conducted using the Luminex xTag Gastroplex (GPP) Analyte Specific Reagent (ASR) [24], designed to detect 16 targets of various pathogens and their toxin genes that are associated with human gastroenteritis. An extra target for detecting the internal control and identifying PCR inhibitory specimens (bacteriophage MS2), was added to the sample prior to extraction. Targets are RNA and DNA of viruses, bacteria and parasites. In this instance, the target pathogens were adenovirus 40/41, *Campylobacter* spp., *Clostridium difficile* toxin A/B, *Cryptosporidum* spp., *Entamoeba histolytica*, *Escherichia coli* O157, enterotoxigenic *Escherichia coli LT/ST, Giardia* spp., norovirus G1/G11, rotavirus A, *Salmonella* spp., shiga-like toxin producing *Escherichia coli*, *Shigella* spp., *Vibrio cholera*, *Yersinia enterocolitica*. The method essentially is a modification of multiplex RT–PCR with Luminex's proprietary universal Tag sorting system on the Luminex xMAP platform. Prior to the GPP procedure, DNA was extracted from stool samples using bioMérieux NucleSense easy MAG extraction system according to the manufacturer' instructions. In brief, 20 μl PCR mix and 5 μl DNA template was added to each tube, then the mixture was vortexed and placed in a thermal cycler. After pre-heating at 53 °C, DNA extracts were loaded and the run was started. Seventy-five microlitres of hybridization mix was added to each well; the plate was sealed and returned to the thermal cycler. The GPP analysis was performed using the Bio-Plex®−200 system (Bio-Rad Laboratories, USA) and Luminex xPONENT® software (Luminex Corp.).

Conventional multiplex using real-time PCR

The elaboration of virulence toxin genes was determined using conventional multiplex real-time PCR. In brief, this incorporated 22 primers for the amplification of 11 virulence genes (*stx1*, *stx2*, *eae*, *bfp*, *LT*, *st11*, *virF*, *ipaH*, *aaFII*, *daaE*, *uidA*) exhibited by diarrhoeagenic *E. coli* [25].

Statistical method

The expression of toxin genes by multiple antibiotic-resistant *E. coli* was analysed by one-way analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test, using GraphPad Prism 5.01 software (GraphPad Software Inc., USA). The level of statistical difference was set at $P \leqslant 0{\cdot}05$.

RESULTS

Assessment of the enteric pathogen profile in stool of diarrhoea patients in Lagos, Nigeria

The simultaneous detection of 16 gastrointestinal pathogens in 91 diarrhoeal stool samples of adult

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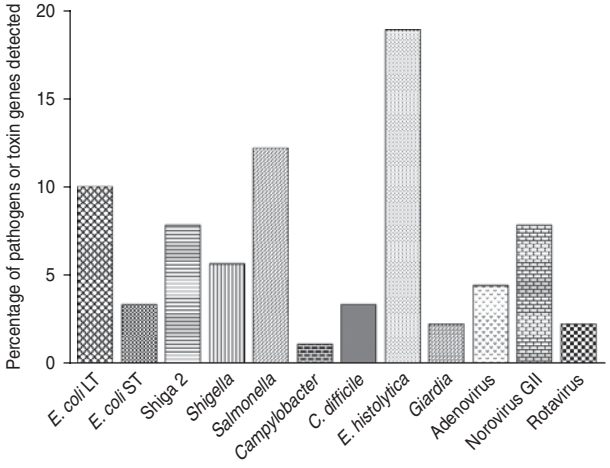

Fig. 1. Percentage of pathogens or virulence toxin genes in adult diarrhoeal stool samples collected from patients in various localities in Lagos, Nigeria. This depicts the simultaneous detection of 16 pathogens using the Luminex xTAG Gastro-intestinal Pathogen Panel (GPP). This figure represents the total of each pathogen type in the whole of Lagos metropolis in Nigeria.

patients in Lagos showed that 12 (75%) out of 16 targeted pathogens were detected. All 11 control stool samples from healthy individuals were negative for the targeted pathogens except *E. coli* which were detected in two samples. Figure 1 represents the detection rate of pathogens. *Ent. histolytica* was the most frequently detected pathogen (19%), followed by enterotoxigenic *E. coli* represented as *E. coli* that expresses heat labile toxin genes (*LT*) and heat stabile toxin genes (*ST*) totalling (14%). *Campylobacter*, detected once, was the least detected pathogen. Three viruses were detected: norovirus G1/G11 (8%), adenovirus 40/41 (5%), and rotavirus A (3%). Out of 91 stool samples, 50 contained pathogens of various numbers, 31 samples had one pathogen, while 19 samples contained two or more pathogens.

Simultaneous determination of the enteric pathogen profile in stool of diarrhoea patients from individual localities in Lagos metropolis

The stool pathogen profile in seven localities in Lagos metropolis was individually assessed. Alimonsho stool samples revealed a pathogen profile pattern in which *Campylobacter* was the most common pathogen (Fig. 2*a*), while in the Shomolu area, *E. coli* (*E. coli LT* and *ST* taken together) and *Giardia* were the most common pathogens detected (Fig. 2*b*). The detection of pathogens in the Oshodi locality (Fig. 2*c*) was very sparse. In instances where a pathogen was detected, only one sample was positive. The reason for the Oshodi data is unclear, but it is noteworthy that cost related non-compliance with dosing may not be common in Oshodi, where most inhabitants being traders can afford self-medication cost. In Surulere (Fig. 2*d*), *Ent. histolytica* and *E. coli* (*LT* and *ST*) were detected along with *Salmonella*, adenovirus and norovirus. In other localities (Yaba, Ikeja, Mushin; Fig. 2*e–g*, respectively), the pattern was one in which *E. coli ST* (Fig. 2*e*), or *LT* (Fig. 2*e–g*), or shiga 2 (Fig. 2*e, f*) were detected, although in scanty proportions.

Detection of toxin genes expressed by *E. coli* isolated from accessible drinking-water sources in Lagos and Wisconsin, USA

Arguably, the frequency of detection of *E. coli* and some of its associated toxins during stool pathogen profiling in Figures 1 and 2, may reasonably be a reflection of a the relationship between toxigenic *E. coli* distribution in accessible drinking water and the clinical disease of diarrhoea; therefore, we decided to examine the expression of *E. coli* toxin genes in accessible drinking-water sources in Lagos and compare it with similar water sources from Wisconsin, although not usually ingested water sources, since the accessible drinking water in Wisconsin is better treated. Results (Fig. 3*a, b*) show that Lagos isolates expressed toxin genes [*eae* (42%), *LT* (10%), *stx2* (6%), *bfp* (5%), *stx1* (4%), *ST11* (2%), v*irF* (1%), *aaFII* (1%), *daaE* (1%)] of biological significance, while Wisconsin water isolates (although not usual drinking water) expressed higher levels of *stx1* (14%) and *stx2* (25%) toxin genes which may be of biological significance; but lesser levels of *eae* (35%) and *LT* (2%). The biomolecule *uidA* is characteristically expressed in *E. coli*.

Detection of toxins elaborated by *E. coli* isolated from adult diarrhoeal stool samples in Lagos and Wisconsin

Based on the finding that *E. coli* isolates from accessible drinking-water sources in Lagos elaborated toxins of concern in diarrhoea, we decided to investigate the possible elaboration of the same profile of toxins in diarrhoeal stool samples from Lagos and determine if our findings could represent a pattern illustrating a

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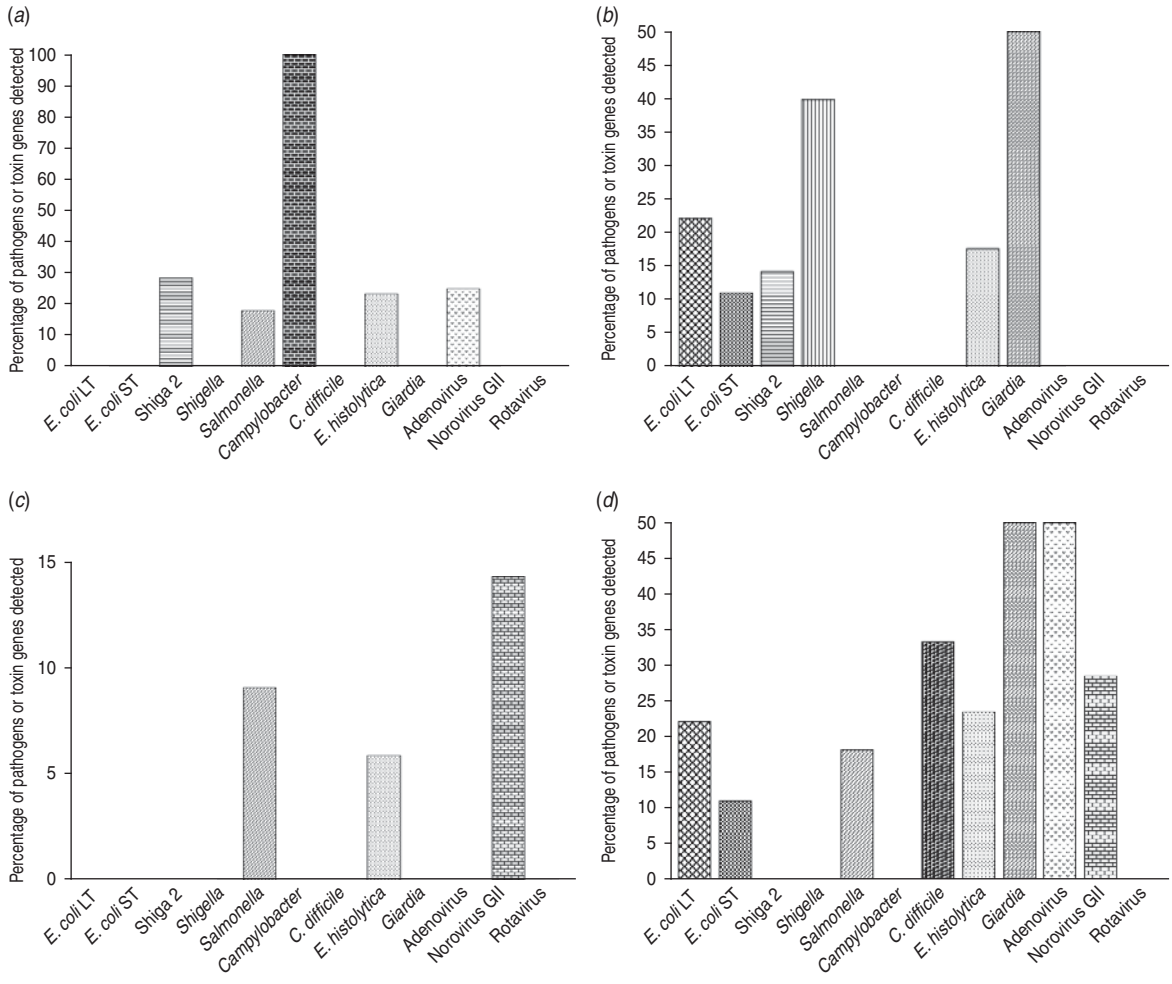

Fig. 2. See the following page for legend.

correlation between toxins of accessible drinking sources and those of diarrhoeal disease sources. Results (Fig. 4*a*) show an expression of toxin genes [*eae* (78%), *LT* (18%), *stx2* (3%), *bfp* (11%), *stx1* (3%), *ST11* (4%), *daaE* (2%)] from *E. coli* isolates which may be related to those from accessible drinking-water sources (Fig. 3*a*). To determine if this pattern of relatedness between water sources and diarrhoeal disease sources can be replicated in Wisconsin, we assessed the expression of toxin genes by *E. coli* isolates from diarrhoeal stool samples in Wisconsin (Fig. 4*b*). While *E. coli* isolates from water in Wisconsin averagely expressed toxin genes *eae*, a higher expression was obtained in stool (54%) and *LT* was unexpressed (Fig. 3*b*). Wisconsin stool isolates expressed significantly high levels of *stx2* (50%), and *stx1* (27%), suggestive of the fact that diarrhoeal disease in Wisconsin may be related to water to an appreciable extent.

Pattern of virulence toxin gene expression by diarrhoeagenic *E. coli* strains that are resistant to various antibiotics

In order to investigate if the antibiotic susceptibility pattern of *E. coli* isolates bear any relationship with toxin elaboration, we decided to determine toxin gene expression by antibiotic-resistant diarrhoeagenic *E. coli*. Table 1 shows the resistance patterns to 16 antibiotics and the expression of toxin genes by *E. coli* strains from diarrhoeal stool samples, while Figure 5 shows the expression of *E. coli* toxin genes and the extent (%) of resistance to multiple antibiotics. The *stx1* toxin gene was detected in strains resistant to 13 (85%) of the antibiotics used. It was not elaborated by isolates resistant to ceftriaxone, cefixime and gentamycin. The *stx2* gene was expressed by isolates resistant to 11 (69%) of the antibiotics used and was not expressed by isolates resistant to ciprofloxacin,

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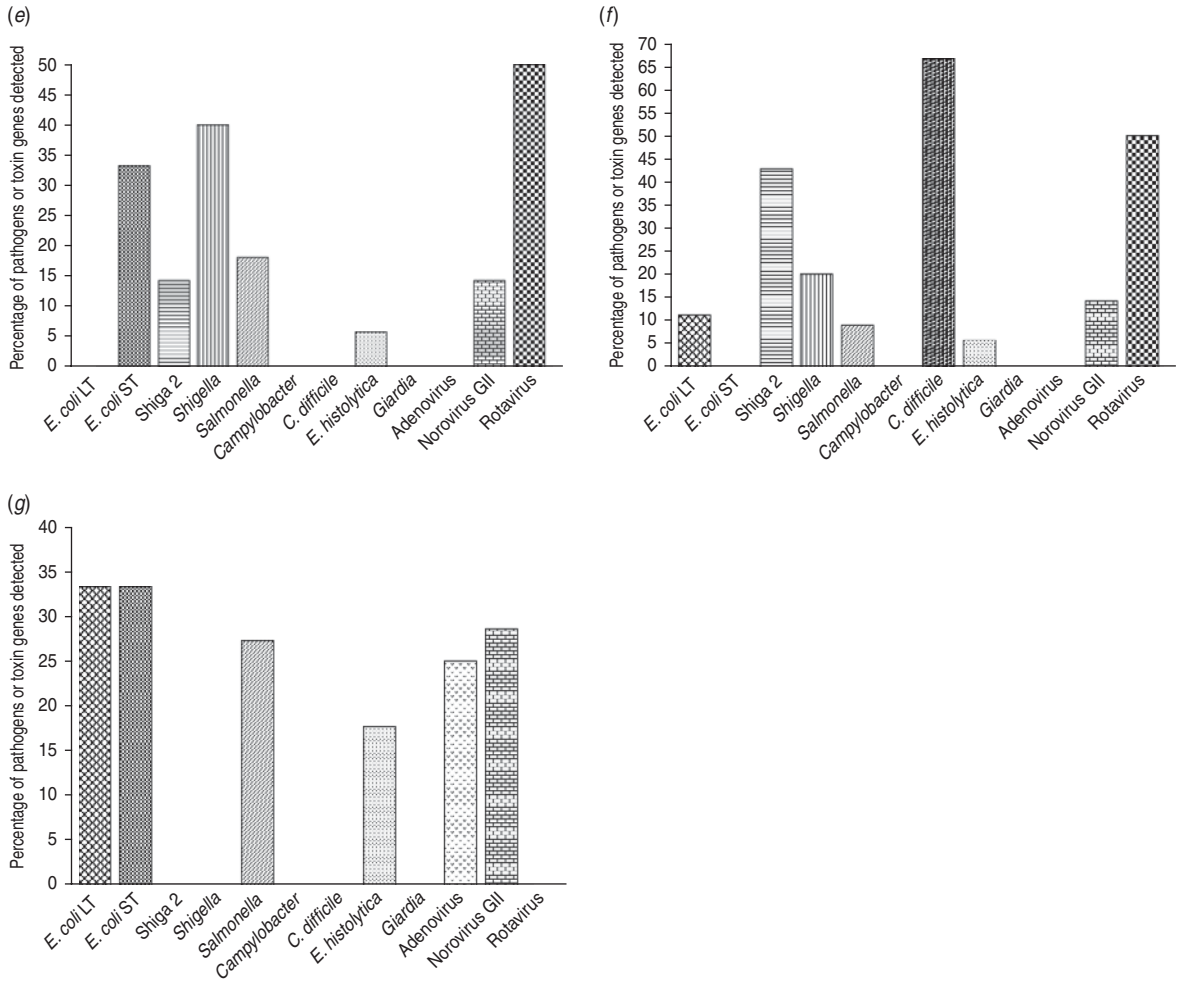

Fig. 2. Percentage of pathogens or virulent toxin genes in adult diarrhoeal stool samples. The stool pathogen profile from diarrhoea patients from individual localities in Lagos, Nigeria is shown: (*a*) Alimonsho; (*b*) Shomolu; (*c*) Oshodi; (*d*) Surulere; (*e*) Yaba; (*f*) Ikeja; (*g*) Mushin.

ofloxacin, norfloxacin, ceftriaxone and gentamycin. The *eae* toxin was identified in strains resistant to 15 (93·75%) antibiotics used. It was not detected in nitrofurantoin resistance. The *bfp* toxin gene was identified in isolates resistant to seven (43·75%) antibiotics. It was not detected in those resistant to floroquinolones (ciprofloxacin, ofloxacin, norfloxacin), nalidixic acid, ceftriaxone, cefuroxim, ceftazidime, nitrofurantoin and amoxcillin/clavulanic acid. The *LT* toxin gene was detected in isolates resistant to all (100%) the antibiotics used. The *ST11* toxin gene was detected in isolates resistant to six (37·50%) antibiotics. It was not detected in those resistant to cephalosporins (ceftriaxone, cefixime, cefuroxime, ceftazidime, except cefaclor), ciprofloxacin, nalidixic acid, amoxycillin/clavulanic acid, nitrofurantoin and gentamycin. The *virF* toxin gene was found in the strains resistant to four (25%) antibiotics. It was not identified in those resistant to floroquinolones (ciprofloxacin, ofloxacin, norfloxacin), the cephalosporins (ceftriaxone, cefixime, cefuroxime, ceftazidime, cefaclor), nalidixic acid, nitrofurantoin, amoxycillin/clavulanic acid, and gentamycin. The *aaFII* toxin gene was detected in isolates resistant to three (18·75%) antibiotics. It was not detected in those resistant to floroquinolones (ciprofloxacin, ofloxacin norfloxacin), cephalosporins (ceftriaxone, cefixime, cefuroxime, ceftazidime, except cefaclor), nalidixic acid, tetracycline, streptomycin, fluoroquinolones, nitrofurantoin, amoxycillin/clavulanic acid, gentamycin. The *daaE* toxin gene was detected in isolates resistant to eight antibiotics (50%). There was no *daaE* toxin indicated in isolates resistant to ceftriaxone, tetracycline, cefuroxime, ceftazidime, streptomycin, nitrofurantoin, amoxicillin/clavulanic acid, and gentamycin.

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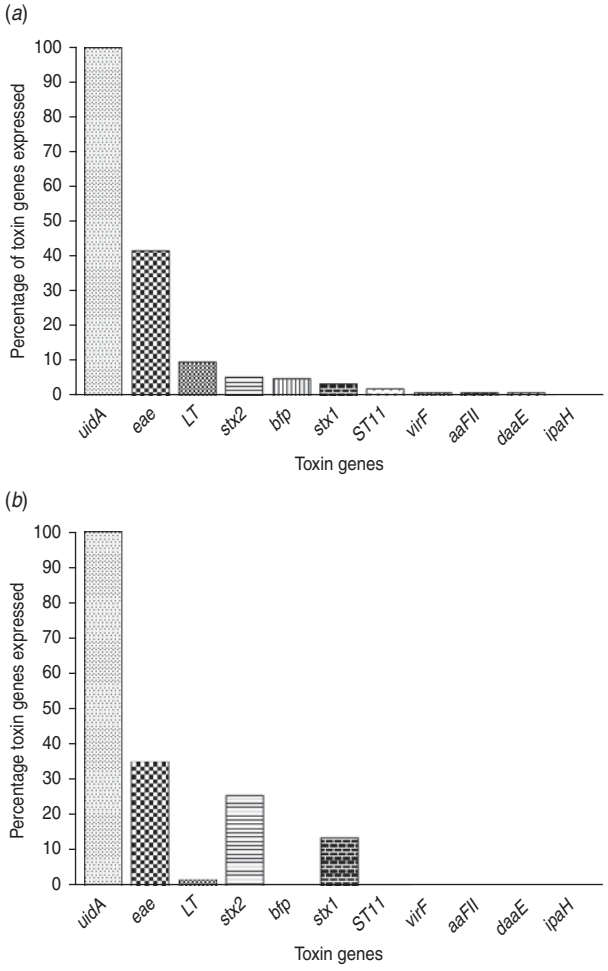

Fig. 3. Virulence toxin genes expressed by *Escherichia coli* isolates obtained from water sources. (*a*) The detection of toxin genes of *E. coli* isolates from accessible drinking water (canal, well, municipal pipe borne and sachet packed water) in Lagos, Nigeria. (*b*) Toxin genes of *E. coli* detected in water sources (beach, storm and treatment plant water) in Wisconsin, USA.

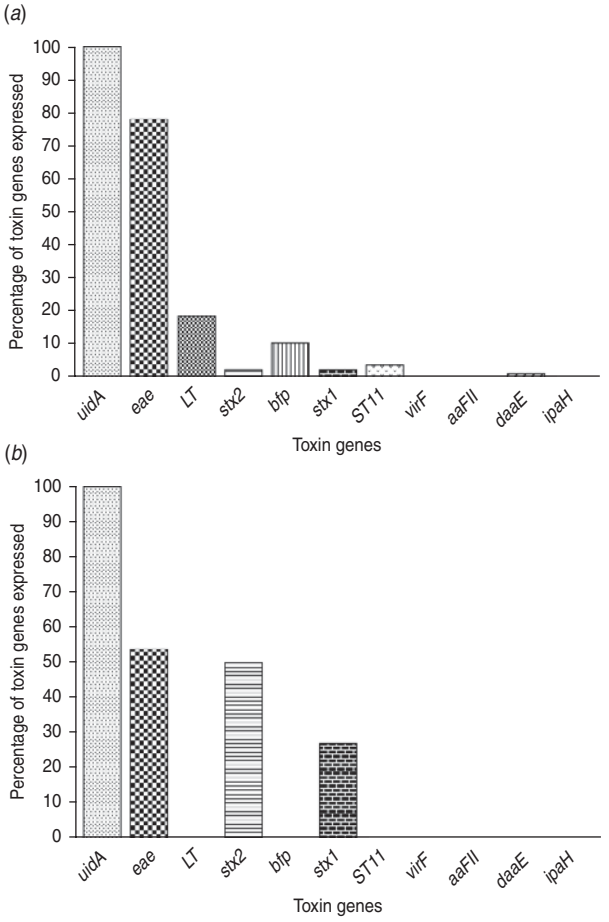

Fig. 4. Virulence toxin genes of *Escherichia coli* isolated from stool strains. The detection of toxin genes expressed by *E. coli* isolated from adult diarrhoeal stool in Lagos metropolis in (*a*) Nigeria and (*b*) Wisconsin, USA is shown.

DISCUSSION

It is increasingly obvious that a wide array of bacterial, viral and parasitic pathogens are capable of infecting the intestine with or without overt dehydrating diarrhoea burden. Although diarrhoeal disease is worldwide in distribution, developing countries are more impacted by the disease burden. The rate of mortality in many countries is on the decline while the morbidity rate remains high [5]. Arguably, the morbidity burden of diarrhoea is accounted for by the systematic malnutrition caused by persistent diarrhoea and chronic enteropathy arising from recurring enteric infections. In developing countries, the problem of diarrhoea is related to poor sanitary conditions as observed in the standard of hygiene in food and water consumed that are often contaminated with an array of enteric pathogens. Thus, 1/6 people (1·1 billion individuals) have no source of safe water and 4/10 (2·6 billion individuals) lack even pit latrines (which on its own is a source of pathogen distribution during flooding), which results in numerous enteric infections and worsening the rates of morbidity from diarrhoea [26]. The burden of diarrhoeal disease on the developing world is tremendous when the malnutrition effect on infants and children is considered. Furthermore, infection with specific enteric pathogens such as enteroaggregative *E. coli* (EAEC) and *Cryptosporidium* spp. can negatively impact growth even in the absence of overt diarrhoea [27–30].

Early detection of the wide array of pathogens involved in diarrhoea would contribute to intervention and control strategies. There are points of intervention

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Table 1. *The expression of virulence toxin genes by antibiotic-resistant diarrhoeagenic* Escherichia coli *from stool samples in Lagos, Nigeria*

	Antimicrobial agents															
Toxin gene	CIP	NA	CRO	CFM	TE	CXM	SXT	CAZ	S	F	OFX	AMC	CN	CEC	AMP	NOR
stx1	+	+	–	–	+	+	+	+	+	+	+	+	–	+	+	+
stx2	–	+	–	+	+	+	+	+	+	+	–	+	–	+	+	–
eae	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
bfp	–	–	–	+	+	–	+	–	+	–	–	–	+	+	+	–
LT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ST11	–	–	–	–	+	–	+	–	+	–	–	–	–	+	+	+
virF	–	–	–	–	+	–	+	–	+	–	–	–	–	–	+	–
ipaH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
aaFII	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+	–
daaE	+	+	–	+	–	–	+	–	–	–	+	–	–	+	+	+
uidA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+, Resistant; –, sensitive.

The antibiotics assessed were ciprofloxacin (CIP); nalidixic acid (NA); ceftriaxone (CRO); cefixim (CFM); tetracycline (TE); cefuroxime (CXM); sulfamethoxazole/trimethoprim (SXT); ceftazidime (CAZ); streptomycin (S); nitrofurantoin (F); ofloxacin (OFX); amoxicillin/clavulanic acid (AMC); gentamicin (CN); cefaclor (CEC); ampicillin (AMP), norfloxacin (NOR).

The toxin gene expression assessed were shiga-like toxin (*stx1* and *stx2*), intimin-*E. coli* attaching and effacing (*eae*), bundle forming pilus (*bfp*), heat labile toxin (*LT*), heat stabile toxin 11 (*ST11*), transcriptional activator virulence gene (*virF*), invasive plasmid antigen H (*ipaH*), aggregative adherence fimbriae II (*aaFII*), diffuse adherent (*daaE*), *β*-glucuronidase (*uidA*).

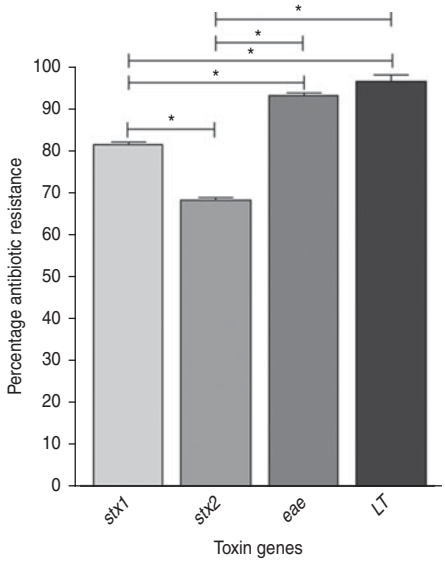

Fig. 5. Toxin gene expression by *Escherichia coli* and the extent (%) of antibiotic resistance exhibited. The *E. coli* isolates that expressed *stx2* genes exhibited a significantly lower percentage of multiple antibiotic resistance compared to those that expressed *stx1*, or *eae*, or *LT* toxin genes. * $P \leqslant 0{\cdot}0001$.

that can lead to the annulment of the cycle of diarrhoea and its burden; these include the advent of new molecular probes that increasingly reveal important viral, bacterial, and parasitic enteric pathogens and their virulence traits and the addressing of the long-term impact of diarrhoea on development and productivity. The use of the Luminex xTAG GPP, enabled multiplex detection of multiple gastrointestinal pathogens and toxins simultaneously in one stool sample as depicted in Figure 1. In this way, the role of multiple infections in the complication of diarrhoeal disease and the impact on disease severity is revealed. The use of xTAG GPP has gone a long way in showing that the gastrointestinal pathogenic burden in a complex city such as Lagos can be analysed creating room for effective infection control and proper antimicrobial therapy. The xTAG GPP is a variant of multiplex which incorporates culture independence, sensitivity and specificity thus having an enormous prospect as a screening tool in routine clinical testing and public health diarrhoea outbreak investigations. The importance of xTAG GPP becomes very obvious when one considers the fact that the current culture- and non-culture-based molecular methods have a much shorter turnaround time.

The detection of a wide range of pathogens in diarrhoeal stool samples from Lagos, Nigeria is important, and of special significance is the detection of some *E. coli* toxin genes in the xTAG GPP procedure (Figs 1, 2*a–g*, respectively). Therefore, it was imperative to further investigate the genes expressed by

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E. coli isolates from stool and water sources (Figs 3*a, b*, 4*a, b*). The intent here is to determine the link between toxigenic strains of *E. coli* from stool and accessible drinking-water sources. The *E. coli* isolates from Lagos were found to express genes that could lead to elaboration of toxins that are highly pathogenic and thus, a serious threat to public health. The expression of the *eae* toxin gene, which encodes for intimin, an intestinal adherence factor, is widely distributed in Lagos. Of interest is the fact that eight diarrhoeagenic *E. coli* virulence toxin genes (*uidA*, *eae*, *LT*, *bfp*, *stx2*, *stx1*, *ST11*, *daaE*) were detected from the Lagos diarrhoeal stool samples (Figs 2*a–g*, 4*a*) while nine virulence toxin genes (*uidA*, *eae*, *stx2*, *stx1*, *virF*, *aaFII*, *bfp*, *ST11*, *daaE*) were detected in accessible drinking-water isolates (Fig. 3*a*), thus the Lagos water isolates had more toxin genes expressed compared to the stool isolates.

The Wisconsin, USA water isolates expressed comparatively less *eae* and *LT* toxin genes (Fig. 3*b*) and the stool isolates expressed significantly high levels of *eae*, *stx2*, *stx1* toxin genes (Fig. 4*b*) apart from the *uidA* toxin genes expressed in either cases. The emerging facts in the comparative assessment of the virulence toxin gene expression between Lagos and Wisconsin isolates are that *stx1* and *stx2* toxins (associated with enterohaemorrhagic *E. coli* activities) were expressed more frequently in Wisconsin water isolates than Lagos, a fact which may not be unconnected with the water sources. It is noteworthy that despite the similarities between the Wisconsin and Lagos water sources studied, in Wisconsin, water from these sources are not drinking water, although it is arguable that they may be accidentally consumed. Moreover, it is reasonable to assume that for the purpose of consumption, the Lagos sources may be subjected to relative filtration which may not significantly affect bacteria load; therefore the presence of enterohaemorrhagic *E. coli* toxins may be more impactful in Lagos than Wisconsin. The high levels of toxin gene (*stx1* and *stx2*) expression in Wisconsin diarrhoeal stool points to the fact that samples were obtained from acutely ill diarrhoea patients compared to Lagos where patients had chronic diarrhoea. Taken together, the Lagos studies show relatedness between water as a pathogen source and the onset of clinical diarrhoea.

There is compelling evidence of the indiscriminate use of antibiotics in the city of Lagos; therefore, there is predisposition of antibiotic resistance. The public health implication of this, alongside the finding of extensive toxin gene expression by *E. coli* isolates both in environmental and stool samples is that an additional burden is borne by patients afflicted by antibiotic-resistant *E. coli* which also expresses virulence toxin genes. This is significant, since among the bacterial agents of diarrhoeal aetiology, diarrhoeagenic *E. coli* occupies a central place. It is therefore imperative that the combination of two burdens (antibiotic resistance and toxin elaboration) calls for strict public health measures that will ensure discipline in therapeutic interventions that involve the use of antibiotics. For instance, it was observed that *eae*, *LT*, and *stx1* and *stx2* (virulence factors of shiga toxin-producing *E. coli*), were the most preponderant toxin genes expressed by antibiotic-resistant Lagos isolates (Table 1). Arguably, more severe cases of diarrhoea will be evident in those localities where the combined burden of drug resistance and toxin elaboration co-exist. Insights into the statistical relevance of multiple antibiotic-resistant *E. coli* isolates to *eae*, *LT*, and *stx1* and *stx2* toxin gene expression (Fig. 5), show the following toxin gene expression pattern $stx2 < stx1 < eae < LT$. Although the approach reflecting the combined burden of antibiotic resistance and elaboration of virulence toxin by antibiotic-resistant *E. coli* on public health is a unique outlook, there is evidence suggesting that these factors are of formidable pathogenesis concern. For instance, the capacity to elaborate shiga toxins is a unique pathogenesis asset to *E. coli* and has an important role in causing disease which may be life-threatening, especially if the disease is accompanied by bloody diarrhoea and haemolytic uraemic syndrome [29, 30]. Indeed, the global significance of poor sanitation-based dissemination of infection in low-income countries should not be underestimated. The importance of this can be seen in relation to horizontal gene transfer of virulence factors resulting in the emergence of new pathotypes as evident in the recent case of the shiga-toxin-producing EAEC pathotype thought to be derived from sprouted seeds from Egypt which were involved in a large uraemic outbreak in Germany and Europe in 2011 [31–33].

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DECLARATION OF INTEREST

None.

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