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Research Article

Prevalence of Fluoroquinolone Resistant Enteric Bacteria in Households' Domestic Kitchen Environment in a Nigerian Urban Setting

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Abstract

The investigation was undertaken to ascertain the prevalence of fluoroquinolone (FQ) resistant enteric bacteria in domestic kitchens in an urban environment. Warri town in the Niger Delta region of Nigeria was the urban settlement used. A multi-stage technique with central Warri and 2 bordering localities as primary stages and 20 randomly selected domestic kitchens/primary area as secondary stages was adopted. The swab-rinse method was used to collect samples from floors, plates and utensils in the kitchens and used to inoculate Nutrient agar for heterotrophic plate counts (HPC), and MacConkey and Deoxycholate Citrate agar for isolating enteric bacteria (EB). Susceptibility of EB to 3 FQs, *Ciprofloxacin, Norfloxacin* and *Levofloxacin* was determined by the agar disc diffusion technique while plasmid curing was by the sodium dodecyl sulphate method. HPC was high (4.31 ± 0.34-4.79 ± 0.22 log cfu/cm2) thereby indicating a "fertile ground" for the growth of EB. The EB identified were *Enterobacter, Campylobacter, Escherichia coli, Salmonella, Shigella, Klebsiella* and *Citrobacter* with variations in the number of isolates (28-48 isolates). Prevalence of resistance to FQs were high; it varied with isolates and kitchen sources and it stood at 35.7-71.1, 30.0-73.7, and 30.0-76.5% for *Ciprofloxacin*, *Norfloxacin* and, *Levofloxacin*, respectively. Prevalence of resistance to the FQs was associated with kitchen sources of EB (X2 = 25.18; P = 0.000). Although with low prevalence (3.4-22.2%), plasmid-mediated FQ resistance occurred with a tendency to be higher in Salmonella and Shigella. Thus domestic kitchens can be vulnerable to ingress of FQ resistant bacteria with consequences of horizontal transfer and therapeutic problems with diarrhoeal infections.

Keywords: Enteric Bacteria; Fluoroquinolones; Domestic Kitchens; Antimicrobial Resistance

Introduction

Fluoroquinolones (FQ) are synthetic broad-spectrum antibiotics that were introduced in the 1980s and have been used for the treatment of various bacterial infections [1]. FQ targets the helicases and topoisomerases during transcription and replication thereby inhibiting bacterial growth [2]. FQs kill many bacteria at low minimum inhibitory concentrations [2] hence they remain useful for the treatment of urinary, respiratory, gastrointestinal, urogenital and veterinary infections despite the growing bacterial resistance [3,4]. The mechanism of resistance has been reported to be associated with chromosomal mutation in DNA gyrase and topoisomerase IV and plasmid-borne resistance genes [5,6]. Resistance to FQ is growing and the prevalence depends on the bacterial strains and sources, hospital settings, countries and local control of usage [7-9]. An analysis of the prevalence trend across the world as it concerns Gram-negative bacteria, shows that the rate of FQ resistance tend to be greater than 20% in Europe and North America while it is up to 75% in China and 69% in Tog (sub-Sahara Africa) [10-12].

Occurrence and prevalence of FQ resistant bacteria is predicated on sources of bacteria and locations. This includes hospital settings

[1,4,13] animals [14-15], sewage and surface water [16] and food [17]. However there is paucity of information on the prevalence of FQ resistant bacteria in kitchens despite the knowledge that kitchens are often sources of food-borne infections. The sources of bacteria in kitchens that may include pathogens have been shown to be bioaerosols, food, humans, hand towels, chopping boards, dish cloths, sinks and refrigerators [18,19]. The pathogens among the bacteria can gain access to food and bring about food-borne infections that can be problematic and costly to treat if resistant to FQ and other antibiotics. It is therefore necessary to update information concerning the spread of FQ resistant bacteria by focusing on domestic kitchens to ascertain their prevalence especially in an urban crowded environment where according to Boadi., *et al*. [20], there is "urbanization without development". Warri town in the Niger Delta region was therefore investigated for the presence and prevalence of FQ resistant enteric bacteria in town's household domestic kitchens.

Materials and Methods

Location and source of samples

Warri is a town that hosts several oil prospecting and refining companies in the Niger Delta region of Nigeria hence it is highly urbanised and suitable for the study. A multi-stage sampling technique was adopted with Warri central and 2 bordering areas (Udu and Uvwie) as primary stages. The secondary stages were the randomly selected 20 domestic kitchens from each of the three primary stages bringing it to a total of 60 kitchens for investiagation. Oral informed consent of the kitchen owners was duly obtained while the protocol for the study was approved by the Microbiology Departmental Research Board.

Collection of samples

Swab samples of plates, utensils and floor were collected from the kitchens at fortnightly intervals for a period of 6 weeks. Each of the cotton wool swab sticks were moistened with 1 ml sterile normal saline and used to swab delineated areas on the floor (10 × 10 cm), plates and utensils (2 x 2 to 5 x 5 cm) depending on the sizes of the surfaces. They were immediately transferred to 9 ml sterile normal saline in tubes and transported to the laboratory for subsequent analyses.

Enumeration of total bacteria and isolation of enteric bacteria

At the laboratory, the tubes were shaken vigorously to remove impinged bacteria. Thereafter, 1 ml aliquots were used to inoculate Nutrient agar (NA) in triplicates by pour plate technique for total heterotrophic plate counts (HPC). The colonies were counted after incubation at 37 °C for 24h with a manual colony counter (Gallenkamp). MacConkey agar (MA) and Deoxycholate Citrate agar (DCA) were streaked with the swabs for the isolation of enteric bacteria and incubated at 37 °C for 24-48 hours. After the incubation period, Gram staining was performed on pure colonies of isolated bacteria and characterized using standard biochemical tests following Clinical Laboratory Standards Institute (CLSI) [21] guidelines.

Antimicrobial susceptibility test

In order to evaluate the bacterial resistance to FQ antibiotics, the agar disc-diffusion method according to CLSI [21] guidelines was used. The inoculum used for susceptibility testing was prepared by suspending 4-5 isolated colonies in 5 ml of sterile physiological saline equivalent to a 0.5 McFarland's standard which served as reference in adjusting turbidity of the inoculums [22-24]. This was then used to inoculate Muller-Hinton agar (MHA) (Oxoid Ltd., England) using a sterile cotton swab. Antibiotics disks containing three FQ antibiotics, ciprofloxacin (CFX), norfloxacin (NFX) and levofloxacin (LFX) (Optun Laboratories Nig Ltd) were placed on the inoculated MHA using sterile forceps and incubated at 37 °C for 24 h. The zone of growth inhibition was measured and recorded as susceptible, or resistant based on CLSI, [21] guidelines.

Plasmid curing

Plasmid curing was performed on resistant isolates using a sub-inhibitory concentration of 10% sodium dodecyl sulphate (SDS). Briefly, an overnight broth culture of the isolate was used to inoculate 4.5 ml of nutrient broth. Then 0.5 ml of 10% concentration of SDS was added, and the mixture was incubated at 37°C for 48 h. After incubation, 0.5 ml of the broth was added to a 4.5 ml freshly prepared nutrient broth and incubated at 37°C for an additional 24 h. After curing, antimicrobial susceptibility testing was carried out as previously described. Cured isolates were identified by their failure to grow in the presence of antibiotics, indicating that the resistance genes were carried on the plasmids eliminated by curing.

Data analysis

Log_{10} transformation of the heterotrophic bacterial counts was undertaken and the data were subsequently analysed by descriptive statistics (mean, standard deviation and range). The differences in the HPC with respect to their sources in the kitchen (utensils,

plates and floor) were analysed by analysis of variance (ANOVA). The association between resistance of enteric bacteria to FQs and their sources in the kitchen were analysed by chi square statistics. The proportion of plasmid mediated resistance was determined by subtraction of the resistance levels after curing from initial overall resistance levels.

Results and Discussion

The heterotrophic plate counts (HPC) were generally high irrespective of their sources in the kitchen (Table 1). This suggests poor sanitation and hygiene but does not necessarily indicate the presence of pathogens because no evidence of correlation with pathogens has been reported [25]. For example the floor tended to have significantly higher HPC except in kitchens located in one of the three primary stages of investigation (Table 1), but this was not reflected in the number of enteric bacteria isolated from the floor, plates and utensils. However, the relevance of high HPC to this study is that it indicated a potentially favourable growth environment for enteric bacteria.

*Location of kitchens	Descriptive Statistics	Bacterial counts (log cfu/cm^2) on:			
		Plates	Utensils	Floor	F(P)
Uvwie community	Mean	4.53[a]	4.35[b]	4.79[a, b]	9.45 (0.000)
	SD	0.20	0.41	0.22	
	Min	4.30	3.30	4.40	
	Max	5.00	4.80	5.30	
Udu community	Mean	4.50	4.31[c]	4.63[c]	3.66 (0.032)
	SD	0.30	0.34	0.47	
	Min	4.10	3.60	4.00	
	Max	5.30	4.80	5.90	
Warri central	Mean	4.62	4.38	4.61	2.79 (0.070
	SD	0.29	0.45	0.30	
	Min	4.11	3.00	4.10	
	Max	5.30	4.80	5.30	

Table 1: Occurrence and comparison of heterotrophic bacterial counts from three sources in the kitchen environment.

Sign. diff: [a]P = 0.040; [b]P = 0.000; [c]P = 0.025; *20 kitchens per location.

There were variations in the occurrence of the enteric bacteria isolated from the kitchens as shown in Figure 1. The number of *E. coli* isolates was the highest in the three kitchen sources while *Salmonella* was the least (Figure 1). The presence of *E. coli* in high numbers was not unexpected because it is of gastrointestinal and faecal origin and it is also frequently part of the human normal flora. The enteric bacterial isolates identified were seven genera/species which were recurring in the 60 kitchens albeit, with variation in numbers; and the composition is identical with those isolated in kitchens from other parts of the world, food processing environment and food handlers [26-29]. The constant presence of *E. coli, Salmonella, Shigella* and *Campylobacter*, well known pathogens attributable to faecal contamination, corroborated the poor hygienic practices indicated by the high HPC. Human skin and hands, and contaminated raw food materials, especially fruit and vegetables, brought into the kitchen, are major sources of enteric bacteria usually found in the kitchen environment [26,30] that may be antibiotics resistant.

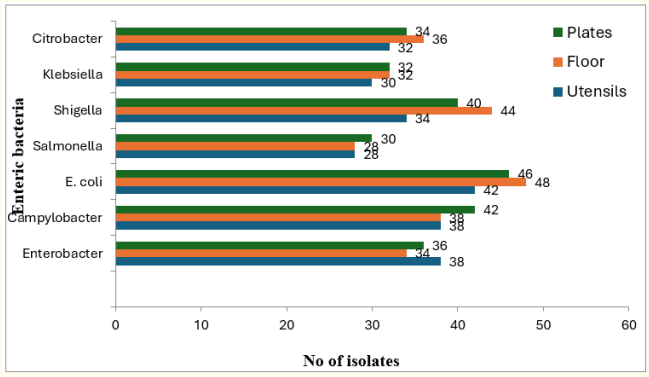

Figure 1: Occurrence of Enteric bacteria in domestic kitchens (N = 60).

The results presented in Table 2 showed that the prevalence of resistance to the three FQs was generally high (30.0-78.9%). This finding substantiates several previous reports on increasing prevalence of FQ resistant bacteria in the environment [1,4,8,9,13]. However, the prevalence patterns varied with bacterial genus/species, kitchen sources and specific FQ antibiotics. When the kitchen plates' isolates FQ resistance were analysed, prevalence of resistance to the 3 FQs among the *Salmonella* isolates was the least (Table 2). On the other hand, *Enterobacter* sp was the most resistant to CFX and NFX while resistance to LFX was most prevalent with *Klebsiella* among the plates' isolates (Table 2).

Table 2: Prevalence of the resistance of kitchen isolates of enteric bacteria to Fluoroquinolones.

Kitchen Sources	Enteric bacteria	n	Prevalence of resistance (%)		
			Ciprofloxacin (CFX)	Norfloxacin (NFX)	Levofloxacin (LFX)
Plates	*Enterobacter*	36	66.7	52.8	63.9
	Campylobacter	42	52.4	52.4	33.3
	Escherichia coli	46	45.7	52.2	32.6
	Salmonella	30	40.0	30.0	30.0
	Shigella	40	47.5	47.5	35.0
	Klebsiella	32	56.2	37.5	65.6
	Citrobacter	34	61.8	50.0	52.9
	All isolates	260	55.1	47.3	45.9
Floor	*Enterobacter*	34	58.8	52.9	76.5
	Campylobacter	38	47.4	55.3	50.0
	Escherichia coli	48	62.5	56.3	75.0
	Salmonella	28	35.7	57.1	50.0
	Shigella	44	36.4	50.0	50.0
	Klebsiella	32	62.5	59.3	59.4
	Citrobacter	36	69.4	52.8	66.7
	All isolates	260	52.4	55.4	60.2
Utensils	*Enterobacter*	38	63.2	63.2	63.2
	Campylobacter	38	71.1	73.7	73.7
	Escherichia coli	42	52.4	57.1	57.1
	Salmonella	28	46.4	60.7	60.7
	Shigella	34	44.1	58.8	58.8
	Klebsiella	30	63.3	56.7	56.7
	Citrobacter	32	65.6	62.5	62.5
	All isolates	242	56.3	61.3	61.3

However, when all plates' isolates were taken together, resistance to CFX was the most prevalent (Table 2). With regards to floor isolates, *Enterobacter*, *Citrobacter* and *Klebsiella* species were the most resistant to LFX, CFX and NFX, respectively while LFX was the most resisted when the totality of isolates is considered (Table 2). Utensils' isolates departed from this trend with *Campylobacter* having the highest prevalence of resistance to all three FQs (Table 2). When all utensils' isolates were taken into account, prevalence of resistance to NFX and LFX were at the same level, but markedly higher than resistance to CFX (Table 2).

It is clear from the foregoing that no clear pattern or trend of prevalence of resistance to FQ could be discerned despite the fact that the enteric bacteria were isolated from the same kitchen environment. This type of differences within an area has been reported in some studies. For example prevalence of resistance to CFX by *E. coli* strains isolated from broiler chicken and broiler farm environment were not the same (Das., *et al*. 2023 [4] and it was also not the same among sheltered companion animals [15]. Source as a factor in the prevalence of FQ resistance was corroborated by the results of the chi square analyses presented in Table 3. Although resistance to two FQs and not the three was associated with source, significant

association involving the three FQs occurred when it was based on the totality of the isolates (Table 3). It should be noted that the bacteria brought into the kitchen via raw food materials, fruits and vegetables may also have originated from different sources before colonising the food materials.

Table 3: Association between resistance of enteric bacteria to fluoroquinolones and their sources in the kitchen.

Fluoroquinolone	Resistance	Kitchen sources and no of enteric bacteria			X^2 (P)
		Utensils	Plates	Floor	
Ciprofloxacin	Resistant	160	162	154	0.96 (0.619)
	Not resistant	124	132	140	
Norfloxacin	Resistant	174	139	163	11.53 (0.036)
	Not resistant	110	155	131	
Levofloxacin	Resistant	189	135	177	26.52 (0.000)
	Not resistant	95	159	117	
All	Resistant	523	436	494	25.18 (0.000)
	Not Resistant	329	446	388	

After curing, the prevalence of resistance to FQ declined by 3.4-22.2% as can be seen in Tables 4. This implied that plasmids were involved in FQ resistance. This finding substantiates the reports on the increasing trend of plasmid mediated resistance to FQ in the environment [18]. Various levels of plasmid mediated FQ resistant bacteria from human, animal, water and sewage sources have been reported by several researchers [1,13,17,31,32] and they range from 0.0 to 25.0% or more The results presented in Table 4 showed that the prevalence of plasmid-borne resistance highly varied and reflected the variations earlier observed in the overall assessment of resistance to the tested FQs. A closer examination of the table showed that *Salmonella* and *Shigella* tended to exhibit more plasmid-mediated resistance than other species. This was not unexpected because *Salmonella* and *Shigella* are the frequent causes of diarrheoa diseases especially in developing countries thereby attracting substantial use of antibiotics; and this can lead to development of resistance than be spread and driven by plasmid-borne genes [33,34].

Implication of findings

The primary objective of this investigation was to ascertain the presence of FQ resistant bacteria in domestic kitchens; and the findings have shown that FQ resistant bacteria are prevalent in the kitchen environment of urban households investigated. Hitherto, the focus of investigations concerning FQ resistant bacteria has been on humans (clinical settings), animals (veterinary medicine), water and wastewater. The spread of FQ resistant bacteria to kitchens is worrisome because of the hazards of gastrointestinal infections that can be difficult to treat. More worrisome is the presence of FQ resistant plasmids especially in *Salmonella* and *Shigella* which are responsible for gastrointestinal infections especially in developing countries. The endemic nature of typhoid fever caused by *Salmonella* in Nigeria [35] can partly be explained by the presence of plasmid-borne antibiotics resistant genes. Thus restricted use of FQs and good sanitary and hygienic habits in kitchens can contain the ingress of enteric bacteria and the spread of FQ resistance.

Study limitation

The objective of the study was to demonstrate the presence and prevalence of FQ resistant enteric bacteria in domestic kitchens. Thus the FQ resistance genes were not investigated and the identification of the enteric bacteria was mostly limited to the genus level. It was also difficult to obtain the consent of domestic kitchen owners for the study hence the number of kitchens investigated was limited.

Kitchen Sources	Enteric bacteria	n	Prevalence of plasmid mediated resistance (%)		
			Ciprofloxacin (CF)	Norfloxacin (NF)	Levofloxacin (LF)
Plates	*Enterobacter*	36	8.3	10.5	8.7
	Campylobacter	42	4.8	9.1	14.3
	Escherichia coli	46	9.5	8.3	13.3
	Salmonella	30	16.7	22.2	22.2
	Shigella	40	10.5	10.5	14.3
	Klebsiella	32	11.1	16.7	9.5
	Citrobacter	34	9.5	11.8	11.1
	All isolates	260	8.6	10.1	10.4
Floor	*Enterobacter*	34	10.0	11.1	7.7
	Campylobacter	38	11.1	9.5	10.5
	Escherichia coli	48	6.7	7.4	5.6
	Salmonella	28	10.0	6.3	7.1
	Shigella	44	12.5	9.1	9.1
	Klebsiella	32	5.0	5.3	5.3
	Citrobacter	36	8.0	10.5	8.3
	All isolates	260	9.1	8.6	7.9
Utensils	*Enterobacter*	38	8.3	8.3	6.7
	Campylobacter	38	3.7	3.6	3.4
	Escherichia coli	42	9.1	8.3	6.7
	Salmonella	28	15.4	11.8	15.4
	Shigella	34	6.7	5.0	5.3
	Klebsiella	30	5.3	5.9	4.5
	Citrobacter	32	9.5	10.0	8.7
	All isolates	242	12.3	7.5	6.9

Table 4: Prevalence of plasmid mediated enteric bacteria resistance to fluoroquinolones.

Conclusion

HPC levels in the kitchen environment were generally high suggesting a good growth environment for microorganisms including enteric bacteria. The enteric bacteria identified from the kitchen surfaces were seven and a substantial proportion of each of them was resistant to the FQs tested. Chromosome-based genes were indicated as mostly responsible for the resistance because the prevalence of plasmid-mediated resistance was markedly lower. By overall assessment, plasmid-encoded FQ resistant genes tended to be more in *Salmonella* and *Shigella* and this can increase the incidence of diarrhoeal diseases. It can be concluded that FQ resistant microorganism were prevalent in the kitchen environment like in clinical, veterinary, and water wastewater settings. Thus treatment of food-borne infections arising from kitchens can be problematic hence the need for restricted use of FQs and good sanitary and hygienic practices in domestic kitchens.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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