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OCCURRENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING *ENTEROBACTERIACEAE* ISOLATES IN COMMUNAL WATER SOURCES IN OGUN STATE, NIGERIA

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RUNNING TITLE: EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING *ENTEROBACTERIACEAE*ISOLATE IN COMMUNAL WATER SOURCES

ABSTRACT

The role of *Enterobacteriaceae* in dissemination and reservoir of antibiotic resistance genes in outbreaks of disease and infections are pressing public health concern. This study is aimed at investigating the antibiotic resistance patterns and ESBL production in water-borne *Enterobacteriaceae* recovered from some selected drinking water sources among 6 towns in Ogun State (Nigeria). Sixty water samples were collected from boreholes and well waters sources, of these samples 68 *Enterobacteriaceae* including *Enterobacter* spp, *Escherichia coli*, *Klebsiella* spp, *Salmonella* spp, *Citrobacter freundii*, *Serratia* spp were recovered and identified presumptively using standard microbiological and biochemical methods. The susceptibilities of the isolates to nine antibiotics were carried out by disk diffusion method and determination of ESBL production was by double-disk synergy method. Of the 68 isolated strains tested, 1 (1.47%) was susceptible to all the antibiotics, 98.5% of the isolates were resistant to ≥ 1 antibiotics and 75% were found to be resistant to ≥ 2 classes of antibiotics. Incidence of water-borne ESBL-producing enteric in this study was 7.14%. The increasing unabated spread of *Enterobacteriaceae* in public water supply harboring resistance genes portends a high risk for communal outbreaks. This necessitates an urgent precautionary and antibiotics surveillance measures in Nigeria.

Keywords: *Enterobacteriaceae*, antibiotic resistance, ESBL

APPARITION DES ENTEROBACTERIES PRODUCTRICES DE BETA-LACTAMASES A SPECTRE ELARGI ISOLEES DANS LES EAUX DE SOURCES DANS L'ETAT D'OGUN, NIGERIA

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TITRE COURANT: ENTEROBACTERIES PRODUCTRICES DE BETA-LACTAMASES A SPECTRE ELARGI ISOLEES DANS LES EAUX DE SOURCES

RÉSUMÉ

Le rôle des *Enterobacteriaceae* dans la dissémination et le stockage des gènes de résistance aux antibiotiques durant les épidémies et infections est un sérieux problème de santé publique. Cette étude a pour objectif, d'examiner les différents modèles de résistance aux antibiotiques et de quantifier la production ESBL chez les *Enterobacteriaceae* d'originehydriqueprésents dans quelques points d'eau potable de 6 villes de l'état d'Ogun (Nigeria).Soixante échantillons d'eau ont été prélevés des forages et des puits. De ceux-ci, 68 *Enterobacteriaceae* comprenant *Enterobacter* spp, *Escherichia coli*, *Klebsiella* spp, *Salmonella* spp, *Citrobacterfreundii*, *Serratia*spp ont été retrouvés et identifiés partiellement, au moyen de méthodes microbiologiques et biochimiques. Les tests de susceptibilités des isolats vis-à-vis de neuf antibiotiques ont été évalués par la méthode de diffusion par disque tandis que le taux de production ESBL a été déterminé par la méthode de synergie double-disque. Des 68 souches isolées et testées, 1 souche (1.47%) était sensible à tous les antibiotiques, 98.5% des isolats étaient résistants à au moins 1 antibiotique et 75% étaient résistants à plus de 2 antibiotiques. L'incidence des bactéries entériques d'origine hydrique et productrices d'ESBL était de 7.14% dans la présente étude. Cette propagation sans cesse croissante des *Enterobacteriaceae* porteuses de gènes de résistance dans les points d'approvisionnement publics en eau potable augure un risque accru d'épidémies communales. Ceci nécessite des mesures urgentes de prévention et de surveillance des antibiotiques au Nigeria.

Mots clés: *Enterobacteriaceae*, résistance aux antibiotiques, ESBL

INTRODUCTION

Enterobacteriaceae is a family of Gram-negative rod shaped bacteria most of which are pathogenic in nature. Notable members of *Enterobacteriaceae* that are commensals of human origin are of clinical significance due to their disease causing abilities [1]. They exhibit a remarkable adaptive capability for acquisition and dissemination of resistance gene such as extended-spectrum beta-lactamase (ESBL) that constitutes one of the currently most widely disseminated and important resistance mechanism that confer resistance to several beta-lactam drugs such as cephalosporins, carbapenems and monobactams [2]. In this current era, antibiotic resistance by bacteria is a leading factor to global therapeutic failure, which is seen as an imminent threat to life and economic loss. Contributory role of enteric bacteria as reservoir to resistance gene and dissemination has been widely reported [3,4]. Colonization of healthy individuals with antibiotic resistant *Enterobacteriaceae*is an instrument in the increase of resistant bacteria at community and hospital level[5]. The presence of enteric bacteria, especially *E. coli* in drinking water regarded as an index of bacteriological quality of water, is an indication of fecal contamination [6]. Studies across Africa have shown that boreholes and well water sources are harboring bacteria of multiple resistance profiles [7,8]. In Nigeria, portable water source are not readily available all over the country.Hence most people depend on alternative water supply from the ground such as boreholes, wells and streams for drinking, cooking and other domestic use. However, while several studies on microbial diversity and total coliform counts from Nigeria underground water supplies have shown that these water sources did not meet international drinking water standards [9−15]. There is insufficient information indicating water-borne enterics as potential reservoirs for antimicrobial resistance genesin this country. This study was therefore carried out to determine the antimicrobial resistance profile and production of ESBL among *Enterobacteriaceae* obtained from various drinking water sources in some towns from Ogun state, Nigeria.

MATERIAL AND METHODS

Study Area

The chosen areas for the study are Ago-Iwoye, Ijebu- Ode, Ilishan, Ikenne, Iperu and Sagamu located at the North-eastern part of Ogun state Nigeria. The areas are characterized by warm frost-free subtropical climate with temperature range of 28 – 33^{o}C and moderate rainfall. Each town is averagely populated with a number of urban development and occupation such as farming, trading and other commercial activities. Available water supply in each place consists of either pipe-borne, borehole, well and streams water or all of the above.

Bacterial Isolate

Sixty water samples consisting of borehole and well waters were collected over a period of 3 months in a sterile sample bottles from the 6 towns (n=10 for each town) in Ogun State. Each collected water samples were cultured directly on MacConkey agar (Lab M Ltd UK) and Eosine Methylene Blue agar (EMB) (Lab M Ltd UK). Presumptive *Enterobacteriaceae* isolates (oxidase-negative facultative aerobic Gram-negative rods) were selected for further studies.

Characterization and Identification of isolates

Further identification of the isolates were carried out by investigation into their various colonial morphology and biochemical reactions such as coagulase, indole, urease, methyl red, voges-prokauer, citrate utilization and sugar fermentation tests as previously described [16] and results were interpreted according to The Bergey's Manual of determinative bacteriology [17,18].

Antimicrobial Susceptibility

Antimicrobial susceptibilities to beta-lactam and non-beta-lactams antibiotics were performed using disc diffusion on Mueller–Hinton agar (Lab M Ltd UK) according to the CLSI guideline [19].*E. coli*ATCC 25922 was used as a control. The antibiotic disks included amoxycillin/clavulanic acid (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), ofloxacin (5 µg), gentamicin (10 µg) andcefixime (5 µg) (Rapids Labs Ltd UK).

Double Disk Synergy Test (DDST)

The isolates with diameter zones of ≤ 18mm for ceftazidime (*n*=28) were further screened for ESBL production by DDST method on Mueller Hinton agar as described by (Jarlier*et al.*,[20] using amoxicillin/clavulanic acid as beta-lactamase inhibitor. Disk containing 30 µg of aztreonam, ceftazidime, ceftriaxone, and cefotaxime, were placed 20 mm apart (center to center) consecutively from the beta-lactamase inhibitor and incubated for 18 - 24 h at 37^{o}C. Synergy between the disks towards the beta-lactamase inhibitor was regarded as presumptive ESBL production [20].

RESULTS

A total of 68 *Enterobacteriaceae*including*Enterobacter*spp (22.06%), *Escherichia coli* (14.71%), *Klebsiella* spp (22.05%), *Citrobacterfreundii* (8.82%), *Salmonella* spp (4.41), *Serratia*spp (1.47) and 26.47% unidentified lactose fermenter oxidase negative Gram-negative bacilli were distributed in water sources across the 6 towns with each organisms showing different antibiotic susceptibilities profiles. Table 1 shows the percentage distribution and respective locations of the *Enterobacteriaceae* isolated in this study.

Ninety-eight and a half per cent (98.5%) were resistant to ≥1 antibiotics while 75% of the isolates were resistant to 2 or more classes of antibiotics tested and the rates of resistance were highest for amoxicillin/clavulanic acid (89.71%) and cefotaxime (76.47%), while highest susceptibilities of 89.70% and 75.00% for most of the isolates was recorded for ofloxacin and ciprofloxacin respectively (Table 2).

TABLE 1: DISTRIBUTION OF *ENTEROBACTERIACEAE* ISOLATES RECOVERED FROM VARIOUS WATER SAMPLES AND LOCATIONS

Locations	No of samples	No of isolates	Percentage (%)
Ago – Iwoye	**10**	**17**	**25.00**
Ijebu Ode	**10**	**11**	**16.20**
Ilishan	**10**	**7**	**10.20**
Ikenne	**10**	**11**	**16.20**
Iperu	**10**	**11**	**16.20**
Sagamu	**10**	**11**	**16.20**
Total	**60**	**68**	**100**

The proportion of water-borne ESBL-producing enteric was 7.14% (2 out of 28 isolates), corresponding to samples recovered from Sagamu borehole water and Ijebu-Ode well water respectively.

TABLE 2: THE SUSCEPTIBILITY PATTERNS OF 68 *ENTEROBACTERIACEAE* AND THE PERCENTAGE (%) RESISTANCE TO 9 ANTIBIOTICS

Antibiotics (Disc potency, µg)	No.and (%) Sensitive	No.and (%) Intermediate	No. and (%) Resistant
Amoxicillin/clavulanic acid (20/10)	**7 (10.29)**	**NA**	**61 (89.71)**
Cefixime (5)	**34 (50.00)**	**1(1.47)**	**33 (48.53)**
Cefotaxime (30)	**1(1.47)**	**15 (22.06)**	**52 (76.47)**
Ceftazidime (30)	**15 (22.06)**	**25 (36.76)**	**28 (41.08)**
Cefuroxime (30)	**3 (4.41)**	**49 (72.06)**	**16 (23.53)**
Ciprofloxacin (5)	**51 (75.00)**	**12 (17.65)**	**5 (7.35)**
Gentamicin (10)	**40 (58.83)**	**7 (10.29)**	**21 (30.88)**
Nitrofurantoin (300)	**48 (70.59)**	**NA**	**20 (29.41)**
Ofloxacin (5)	**61 (89.70)**	**3 (4.41)**	**4 (5.88)**

DISCUSSION

Microbiological assessment of drinking water is the most important because it accounts for the microbial quality and level of contamination that can render the water fit or unfit for human consumption [21]. According to World Health Organization and Nigerian Standard for Drinking Water Quality [6,22] drinking water must not contain any fecal coliform bacteria. In this present study, high percentage members of *Enterobacteriaceae* were detected in the water sources of the towns investigated, suggesting that the available waters in the investigated regions are heavily contaminated with bacteria, which are possibly of fecal origin and could pose a potential public health hazard.

In this study,7 genera of *Enterobacteriaceae* were detected of which *Enterobacter*spp and *Klebsiella* spp had the highest percentage frequency of occurrence (22.06%) followed by *E. coli* (14.70%), *C. freundii* (8.82%), *Salmonella* spp (4.41%), *Serratia*spp (1.47%). There are 18 (26.47%) of the isolated bacteria showing similar biochemical characteristic of the family *Enterobacteriaceae* belonging to different genera that we were unable to identify due to our limited resources. Similar findings on the presence of enteric bacteria of above genera from well water and other water sources have been previously reported in Nigeria [10,12,23,24]. Incidence of these bacteria in portable water is unsafe without treatments and calls for urgent attention because these bacteria are pathogens that have been reported to be associated with serious human infections globally.

From the antimicrobial susceptibility studies, 98.5% of the isolates were resistant to ≥ 1 antibiotics and 75% were found to be resistant to ≥ 2 classes of antibiotics. Previous studies on water enteric bacteria from Nigeria and other African countries also showed high antibiotics resistance profiles and similar rates of antibiotic resistance with those found in this study. For instance, a study in Nigeria found 92.3% of the organisms tested to be resistant to ≥1 antibiotic while 77.3 % were resistant to ≥ 2 antibiotics [23]. In another study by Lin *et al*. [7] from South Africa, 94.7% water-borne enteric isolates were found to be resistant to ≥ 1 antibiotic and 75.2% of the isolates were multidrug resistant suggesting water-borne enterics may have a high level of antibiotic resistance. High-level resistance to beta-lactam drugs (cephalosporins and cephems) was observed among the isolates investigated. Resistance to beta-lactam drugs especially the third-generation cephalosporins and the cephems has been attributed to the presence of extended-spectrum beta-lactamase which are either transferable through mobile genetic elements or could be chromosomally mediated [25]. Studies in Nigeria have shown the consequence of clinical isolates acting as reservoir for transferable antibiotics resistance genes in plasmids and integronsamong Gram-negative bacteria [26,27]. The resistance to amoxicillin/clavulanic acid, cefotaxime, cefixime and ceftazidime by the isolated being 89.71%, 79.47%, 48.53% and 41.08% respectively, suggested the presence of ESBL production among the isolates. Although high resistance to ceftazidime and cefotaxime drug was, the rate of carriage of ESBL by these pathogens by phenotypic assay was low (7.14%). This is in agreement withlow ESBL production (5.3%) among *Enterobacteriaceae* isolated from drinking waterin Central Africa previously reported [28]. In contrast, Soge *et al*. [8] reported a non-resistance to cefotaxime and ceftazidime by water-borne multidrug resistant Gram-negative bacteria isolate from Uganda. Since the genetic basis of resistance to both ceftazidime and cefotaxime was not investigated in this study it is difficult to conclusively suggest the reason for differences in result. However, global reports on outbreaks have established that transmission of pathogenic bacteria by drinking water remains a significant cause of illness. Presence of *E. coli* and other coliform bacteria isolated in this study may have been due to contamination from human fecal discharges as widely observed elsewhere.

In conclusion, presence of pathogenic bacteria suspected to harbor resistance genes in drinking water samples investigated in this study pose a greater risk of community acquired infections which could lead to disease outbreaks and/or transfer of drug resistant bacteria to human. Consumption of such water could contribute to the dissemination and persistence of antibiotic resistant bacteria constituting a public health hazard. The low level detection of ESBL among the cephalosporin resistant isolates suggests an interplay of other resistance genes especially ESBL that are not easily detected using conventional DDST method as used in this study. Further studies to determine the types of resistance genes present in the isolates are ongoing in our laboratory.

Conflicts of Interest

The authors declared no conflict of interest.

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