Frontiers in Environmental Microbiology
2022; 8(3): 55-61
http://www.sciencepublishinggroup.com/j/fem
doi: 10.11648/j.fem.20220803.12
ISSN: 2469-7869 (Print); ISSN: 2469-8067 (Online)

Freshwater Linked Resistance Profile and Prevalence of Escherichia Coli Producing ESBL Type CTX-M Strains in Cameroon Urban Cities

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To cite this article:

Cecile Ingrid Djuikoue, Farid Wega, Audrey Limou Dayomo, Cedric Dylan Seugnou Nana, Dorine Ngatcheu Ekeu, Celianthe Guegang Guegang, Dana Djouela Djoulako, Kensese Sontin Mossanda, Venant Tchokonte Nana, Omer Njajou. Freshwater Linked Resistance Profile and Prevalence of Escherichia Coli Producing ESBL Type CTX-M Strains in Cameroon Urban Cities. *Frontiers in Environmental Microbiology*. Vol. 8, No. 3, 2022, pp. 55-61. doi: 10.11648/j.fem.20220803.12

Received: August 9, 2022; **Accepted**: August 26, 2022; **Published**: September 5, 2022

Abstract: Mutualistic human digestive bacteria resistant to antibiotics released into the environment have negative effects on health. The aim of this study was to describe the presence of Escherichia coli (E. coli) strains producing extended spectrum β-lactamases of the CTX-M type strains in three urban cities of Cameroon. A total of 79 E. coli strains resistant to cefotaxime were isolated from 200 freshwater samples collected and inoculated in EMB medium supplemented with cefotaxime. Isolated colonies were subjected to an antibiogram according to CA-SFM 2019 and the risk factors associated with their prevalence were also assessed. The results showed that 51% of samples were positive for E. coli CTX-M, a large portion of which (77.4%) produced an extended spectrum β-lactamases-CTX-M type. Meanwhile, only 59% of strains were resistant to β-lactamases. A co-resistance to cotrimoxazole (81%), aminoglycosides (57.3%), quinolones (43%), and fluoroquinolones (17.7%) has been reported. Interestingly, E. coli resistant to cefotaxime strains was influenced by the points of collection, latrines proximity and human activities. A multivariate analysis indicated 39.5% of E. coli ESBL CTX-M strains prevalence associated with proximity to latrines and human activities. The Increasing E. coli ESBL CTX-M strains prevalence in Cameroon is indeed a public health concern. Therefore, a "One Health Approach Operationalization Program" that would include sensitizing the population and improving sanitation interventions in effective waste management, is crucial in maintaining a healthy environment.

Keywords: Surface Water, Escherichia Coli, Esbl Type Ctx-M, Antibiotics, Risk Factors

1. Introduction

Fresh water contamination represents a significant source of digestives and systemic diseases (Cholera, Typhoid, Hepatitis A and B) and millions of deaths with over 70% in developing countries [1, 2]. Poor access to drinking water, poverty, urbanization and high density of population, absence or deterioration of infrastructure and weak drainage system of water supply remain major obstacles and health burden in most developing countries [3, 4]. These bottlenecks compelled citizens to seek for alternative sources of water from lakes, rivers, wells, and other means, that may lead to

high exposure to a variety of pollutants from various anthropic sources, including agropastoral, industrial, domestic, environmental and even from hospital waste [5-7].

Despite its great potential to strengthen collective action across sectors, One Health implementation is still poorly used in advancing knowledge and decisions making in waste management, and antimicrobial appropriate uses in infectious diseases prevention and control. Fresh waters contain multiple pollutants and might become favorable sites of biological and chemical pollution [8, 9]. The draining of antibiotics residuals at varied concentration from hospital waste into fresh water also constitutes source of emergence and spread of resistant pathogens (100 to 2000 ng/L) [10-12]. Pollutants could be bacteria like Escherichia coli (E. coli), where their presence in aquatic environment can produce chronic diarrhea diseases in freshwater users or consumers [12, 13]. It has been reported that the interaction between bacteria and antibiotics could lead to the development of adaptational resistance mechanisms, such as Enterobacteria producing extended spectrum β-lactamases (E-ESBL) [14].

Nowadays, E-ESBL strains are multiresistant to several antibiotics in different settings [15]. Previous studies have reported the presence of ESBL CTX-M types E. coli multiresistant strains isolated from urinary tract infections and fresh waters environments [16]. CTX-M phenotype is principally expressed by E. coli in aerobic intestinal microbiota in Human (106 à 1011 UFC/g in feces) [11, 14]. Hence, ESBL CTX-M types E. coli strains carriage can excrete or eliminate every day about 1010 UFC/g in feces [17]. The emergence and spread of resistance and multiresistant bacterial strains in freshwater and aquatic environment represent a serious public health challenge.

The prevalence of ESBL CTX-M types E. coli strains in fresh and aquatic environment seems alarming but little is documented on its effects on health over time, with an estimated over 110 million reservoirs in Africa [11]. According to the World Health Organization [18], ESBL CTX-M types E. coli strains constitute 2 to 70% in 13 African countries. For example, in Algeria, fresh waters from drainages were contaminated with 94,1% by ESBL CTX-M types E. coli strains [19]. Another study in West Africa reported that ESBL CTX-M types E. coli strains were found in hospital settings and within communities [20]. Similarly, in Burkina Faso, about 35% of ESBL CTX-M types E. coli strains were reported associated with water infections [21]. Likewise in Congo, 7,4% of ESBL CTX-M types E. coli strains were found in wastewater and 3,3% pre-packed drinking water [3]. A predominant of E coli fecal forms of Enterobacteria was reported in domestic water in Babesi, North West region of Cameroon [22] and in lac water in Nchi, Bamoun plateau in the West region of Cameroon [23]. Yet, little is reported on E. coli strains producing ESBL CTX-M type in fresh water in sub-Saharan Africa and particularly in Cameroon. The aim of this study was to describe the presence of E. coli strains producing ESBL CTX-M type in urban cities in Cameroon.

2. Methods

2.1. Study Design

A cross-sectional and analytic study was conducted from May, 26 to December 26, 2019 in the cities of Yaoundé, Douala and Bafoussam. Fresh water samples were collected from various sites and transported under standard conditions within 4 hours for microbiologic analysis performed in the Microbiology laboratory at the University Clinic des Montagnes (CUM), Bangangte, Cameroon.

2.2. Sample Collection and Collection Sites

Freshwater samples (n= 200) were collected from 3 urban cities namely Douala (n=70), Bafoussam (n=65) and Yaoundé (n=65). This includes freshwater samples used by populations for domestic, professional veterinary and agriculture activities in the areas surrounding the sites. The sites were named 1 to 8; with sites 1-3 in Douala, sites 4-6 in Yaoundé and sites 7 and 8 in Bafoussam. An aliquot of 5mL was sampled in sterile tubes at various spots per sites and per points of waste water discharge. The samples were transported in iced coolers and were brought to Bacteriology Laboratory at CUM for further analysis.

2.3. Bacterial Isolates

Following cellulose membrane filtration (porosity: 0.45 µM and diameter: 55 mM), into 5 mL sterile pot filter, an homogenized solution was used and poured into petri dish containing eosin methylene blue (EMB) and cefotaxime solution (1 mg/L). Isolates of different morphological colony types strains resistant to cefotaxime were observed and counted after 24h incubation at 37°C.

2.4. Bacteria Identification

Escherichia coli strain identification was performed using Api 20 E system, where each gallery was inoculated on bacteria suspension prepared at 0.5 Mc Farland [23]. A total of 102 E. coli strains resistant to Cefotaxime were recorded, and other strains were identified as Klebsiella spp and Enterobacter spp strains.

2.5. Antibiotic Susceptibility Testing and Phenotypic ESBL CTX-M Detection

Antibiotic susceptibility testing on E. coli strains was performed using Kirby-Bauer method. The diffusion method on Muller Hinton gel was performed according to CA-SFM 2019 recommendations [24]. Antibiotic tests include amoxicillin, amoxicillin + clavulanic acid, cefotaxime, ceftazidime, cefepime, cefoxitin, amikacin, nalidixic acid, imipenem, ciprofloxacin ofloxacin, Fosfomycin, kanamycin, cotrimoxazole, gentamicin and ticarcillin. The diameter of inhibition was observed as the result of antibiotic resistance. Broad spectrum Beta-Lactamase (ESBL) type CTX-M from E. coli strains was shown by the synergism image characteristics of cefotaxime (C3G) and beta lactamase inhibitor

(Clavulanate acid + Amoxicillin).

2.6. Data Analysis

All variables were collated in Microsoft Excel 2019 and analyzed using Statview software, version 5. Variables including presence or absence of E. coli and ESBL CTX-M types E. coli strains in freshwater; diameter of inhibition for resistance and co-resistances to other antibiotics families were documented. Risk factors were also analyzed using logistic multivariate regression. P value less than 0.05 was considered statistically significant, with confidence interval at 95%.

3. Results

3.1. Identification of Bacteria in Different Samples in Selected Towns in Cameroon

Our results (Table 1) showed that 102 out of 200 (51%) E. coli was the most predominant bacteria in freshwater contaminated with fecal waste. Out of which 24.5% of the contamination was in Douala, 14% in Yaoundé and 12.5% in Bafoussam. The other contaminations found were Klebsiella spp and Enterobacter spp strains in the proportion of 32% and 11.5%, respectively.

Table 1. *Distribution of identified bacteria in urban cities.*

Species	Escherichia coli n (%)	Klebsiella spp n (%)	Enterobacter spp n (%)
Yaoundé	28 (14)	32 (16)	7 (3.5)
Douala	49 (24.5)	15 (7.5)	11 (5.5)
Bafoussam	25 (12.5)	17 (8.5)	5 (2.5)
Total	102 (51)	64 (32)	23 (11.5)

3.2. Prevalence of ESBL CTX-M Types E. Coli Strains

The prevalence of ESBL CTX-M types E. coli was 77.4% (79/200) and was predominant (35.7%) in all the 5 study sites (Table 2).

Fresh water containing ESBL CTX-M types E. coli strains were reported in sites 2 and 5 at 20% and 35.5%, respectively. However, the running freshwater contained ESBL CTX-M types E. coli strains was identified mainly in site 4, with about 17.1%. Interestingly, the Municipal lac in Yaoundé recorded low prevalence of E. coli ESBL CTX-M strains (4.6%) (Table 2).

Table 2. *Distribution of E. coli ESBL CTX-M strains based on site of collection.*

Sites	E. coli ESBL CTX-M	
	Total (n)	Percentages (%)
Yaoundé		
Site 1	03	4.6
Site 2	13	20
Site 3	6	9.2
Douala		
Site 4	12	17.1
Site 5	25	35.7
Site 6	8	11.4
Bafoussam		
Site 7	9	13.8
Site 8	03	4.6
Total	79	100

Site 1: municipal lac; site 2: Damas area; site 3: Mfoundi fesh river; site 4: Bonabéri river; site 5: Ndogbong Lake 1; site 6: Ndongbong Ndogbong Lake 2; site 7: Damté river; site 8: Rond-point Boulevard river.

Table 3. *Risk factors associated with E. coli ESBL CTX-M presence in freshwater surfaces using univariate analysis.*

Variables	Total (N)	*E. coli* ESBL CTX-M prevalence		P-value
		(N)	(%)	
City of collection				
Bafoussam	65	12	19.3	Ref
Douala	70	45	64.2	0.01
Yaoundé	65	22	34	0.40
Proximity to latrines				
No	89	9	10.1	Ref
Yes	111	70	63	0.006
Site of water disposal ≥2				
No	59	18	30.5	Ref
Yes	141	61	43.2	0.05
Anthropic activities				
No	65	12	18.4	Réf
Yes	135	67	49.6	0.03

Overall, an average of 21,4% prevalence of contamination was observed in all Douala sites compared to other sites located respectively in Yaounde (11.2%) and Bafoussam (9.3%). Remarkably, high prevalence of contamination (35.7) was observed in site 5 from Douala.

3.3. Evaluation of E. coli ESBL CTX-M Susceptibility Testing and Resistance Profile to a Range of Antibiotics

Our results from antibiogram showed that E. coli ESBL CTX-M strains had higher and varied degree of resistance to β-lactamases group of antibiotics (88.6% to Amoxicillin, 76% Ticarcillin, 100% to Cefotaxime, 84.8% to Ceftazidime and 44.3% to Cefepime. The aminoside group of antibiotics showed 63.2%, 51.8% and 57% resistance to Gentamicin, Kanamycin and Amikacin respectively, and calls for the need for One Health approach operationalization in tackling antimicrobial resistance epidemics.

Analysis of co-resistance of E. coli ESBL CTX-M strains revealed range of level for quinolones antibiotics: 43%, 17.7%, 21.5% and 14% respectively for Nalidixic acid, Fluoroquinolones, Ciprofloxacin and Ofloxacin. E. coli ESBL CTX-M strains showed higher resistance to sulfonamides mainly Cotrimoxazole (81%) as well as Fosfomycin (53.1%).

Surprisingly, carbapenems which are the latest group of antibiotics used nowadays against Enterobacterial infections showed imipenem resistance of 11.3% on E. coli ESBL CTX-M strains.

3.4. Prevalence and Risk Factors E. Coli ESBL CTX-M Strains in Fresh Water

The prevalence of E. coli ESBL CTX-M strains was reported higher in Douala compared to Yaounde and Bafoussam cities with 64.2%, 34% and 19.3%, respectively.

Overall, the univariate analysis demonstrated that sites of collection of freshwater in urban cities, proximity to latrines, and number of sites of freshwater discharge were higher than 2. The anthropic activities were statistically and significantly (P= 0.01; 0.006; 0.05 and 0.03) associated with freshwater contamination by E. coli ESBL CTX-M strains prevalence. (Table 3), proximity to latrines being the most significant (P= 0.006).

Table 4 shows that proximity to latrines and anthropic activities were statistically significant risk factors of E coli ESBL CTX-M prevalence in the various studied sites (OR= 7.79; IC95% [2.23; 14.03]; P=0.02). Therefore, limiting contamination of household waste, the spatial separation of latrines and surface water is essential to prevent and reduce the spread of resistant bacteria into the environment.

Table 4. *Risk factors associated with E. coli ESBL CTX-M presence in fresh water surfaces using multivariate analysis.*

Variables	OR	IC à 95%	P-value
Presence of E. coli ESBL CTX-M in Douala city			
No	Ref.		
Yes	0.19	[0.87; 29.19]	0.1
Proximity to Latrines			
No	Ref.		
Yes	7.79	[2.23; 14.03]	0.02
Sites of water discharge ≥2			
No	Ref.		
Yes	1.89	[0.4; 9.05]	42
Anthropic Activities			
No	Ref.		
Yes	1.89	[1.4; 4.5]	0.05

OR: Odd ratio; IC à 95%: confidence interval at 95.

4. Discussion

The operationalization of One Health approach interplay in the fight against antimicrobial resistance and the advancement of antimicrobial resistance stewardship is a crucial step in the evaluation and identification of resistance strains mapping. Here, we applied such "One health" approach in assessing the susceptibility profile of Escherichia coli producing ESBL type CTX-M strains isolated from fresh water in 3 urban cities namely, Yaoundé, Douala and Bafoussam, Cameroon.

Our results showed that the prevalence E. coli resistance to cefotaxime was 51% (102 out of 200) samples collected in the 3 selected urban cities. This proportion is less than 72% found by Ouahchia et al [10], while working on the pollution by E. coli strains in running freshwater in Mazafran, Algeria. The difference in these reports could be explained by the ecological aquatic nature receiving diverse pollutants from agriculture, domestic and industrial firms with increasing risk of E coli pollution. However, weak prevalence of fecal enterobacteria mainly E coli strains from domestic water samples was reported by Njoyim et al [22] in Cameroun.

Our findings from susceptibility testing showed that 77.4% E. coli strains produced ESBL of type CYX-M. This result is less than 94.1% prevalence of E. coli ESBL type CTX-M strains reported by Alouache et al [19]. The reason could be due to samples analyzed were from beach sites containing mixture of aquatic and drainage water, waste from diverse sources.

Results from the analysis of the prevalence of E. coli ESBL CTX-M strains based on sites of collection showed that site 5

(pond) was the highest at 35.7%. The high prevalence was related to increasing domestic waste accumulation near freshwater surface. Bagalwa et al [24] showed that the domestic waste hosted diverse pathogens including E. coli multiresistant strains. However, Site 4 had 17.1% of E. coli ESBL CTX-M strains. This result is less than those reported by Rakotovao-Ravahatra et al [25] indicating 42% of E. coli resistant to antibiotics in Seine Bassin river, receiving diverse waste/pollutants or discharge from different sources. Interestingly, the Yaounde Municipality Lake showed less (4.6%) E. coli ESBL CTX-M strains, which suggest less contact with human regular and diverse anthropic activities and limited pollution. This prevalence is less than 94.1% and 36.4% as reported on antibiotic resistance in beach water and multiresistant E. coli ocean sites by Alouache et al. [19] and Toudji et al [26], respectively.

Our results from E. coli ESBL CTX-M strains resistant third generation of antibiotics mainly to β-lactamases at 77,2% to penicillins and 59% to cephalosporins – most used antibiotic in animal and human medicine, and easily accessible in drugs street vendors and chemist off licence shops at competitive prizes. Noteworthy that high prevalence of E. coli ESBLCTX-M strains in samples have the ability to hyfdrolyse β-lactamases mainly penicillins, but not carbapenems. This is consistent with the report of Rakotovao-Ravahatra et al [25] in Madagascar. We also found 11.3% resistant strains to imipenem, which is higher than 2.19% reported by Toudji et al [26], but less than 16.75% reported by Konaté et al [27].

Our results from antibiogram analysis showed 81% co-resistant strains to Co-trimoxazole. This finding is consistent with 88.4% reported by Sbiti et al [28], indicating the persistence and evolution of resistant E. coli strains. The prevalence of Co-resistance to Fosfomycin was 53.1%, aminoxides (63.2% to gentamicin), and quinolones (34% to nalidixic acid). These findings are greater than those reported by Allali et al [29] indicating: aminoxides 38%, Fosfomycin 9%, and quinolones 28%, and was found to be due to over the counter prescription and auto-medication, overconsumption of broad antibiotics spectrum and strains selective pressure in communities. Multi-resistances of E. coli ESBL CTX-M to aminoxides, quinolones, fluoroquinolones and co-trimoxazole was documented and could be explained by genetic resistant plasmid transmission.

Anthropic activities are statistically significant risk factor of contamination E. coli ESBL CTX-M (OR= 1.9; IC95% [1.4; 4.5]; P= 0.05) in multivariate analysis. Also, domestic waste can contain various microbes responsible for contamination and infestation in aquatic environment. Housounou et al [30] in 2016 reported that the sources of pollution of freshwater are generally from anthropic nature. Similarly, Bagalwa et al [24] showed that E. coli ESBL strains in domestic waste could be responsible for diarrheoea diseases. Likewise, Adjagodo et al [31] reported that anthropic activities interfere with freshwater surface leading to health and environmental health issues linked to fecal bacteria and multiresistant antibiotic strains. Another study [32] previously showed that residence proximity to latrines was a statistically significant risk factor of E. coli ESBL CTX-M strains contamination in fresh water (OR= 7.79; IC95% [2.23; 14.03]; P= 0.02). This can be explained by the location of traditional latrines near the sites of sample collection, as water drained directly in the surface water increasing the risk of contamination by fecal bacteria carriage. Furthermore, drainage of soils by erosions and heavy rains contribute to discharge of latrine water in fresh water, which is consistent with the work of Li et al [32] who showed that E. coli ESBL strains in latrines could contribute to E. coli ESBL carriage in water surface in rural areas. Escamilla et al. [33] however, have shown that water samples near latrines, infested ponds and high human population density were more frequently contaminated by multiresistant coliform strains. Thus, latrines are potential source of contamination of freshwater and sites with multiresistant fecal bacteria. These multiresistant strains are indeed the most abundant in latrines [34].

Our findings on proximity to freshwater sites of latrines, anthropic activities as risk factors were statistically and significantly associated with E. coli ESBL CTX-M strains contamination. We reported strains of bacterial abundance and degree of resistance revealed water pollution and multiresistant E. coli ESBL CTX-M carriage, emergence and spread including as consequence of these risk factors and exposure link health and environmental menace. Contextual adoption and implementation of strict hygiene and sanitation measures are required to mitigate and reduce contamination of fresh water by fecal bacteria in these cities in Cameroon.

5. Conclusion

Increasing E. coli ESBL CTX-M strains prevalence is a public health concern in Cameroon. The prevalence of E. coli ESBL CTX-M strains reported in this study was 39.5%, with associated risk factors including the proximity to the latrines and human activities in multivariate analysis. One Health approach operationalization programs including raising population awareness and improved sanitation interventions in effective environment and waste management is crucial to mitigate bacterial infections prevention and control, antimicrobial resistance, and appropriate use of antibiotics. This approach can be of great help to the global health governance (GHG).

Funding Support

This research has been supported by the NGO Prevention and Control [grant number 2020-10002].

Acknowledgements

The authors thanked Dr Tchoukoa Serges, Dr Fotsing Pierre, Mr. Mbianda Peguy, Mr. Deumi for allowing this work to be carried out at the University Clinic des Montagnes.

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