

Antibiotic Resistance Phenotypes of *Enterobacteriaceae* Isolated from Household Wastewater in Brazzaville, Republic of Congo

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Abstract

Household wastewater is a source of pollution and can present health risks when discharged into the environment. Thus, samples of household wastewater from a few neighborhoods in Brazzaville were analyzed for microbiological quality. The various samples were cultured for isolation on solid media using conventional microbiological methods. The bacteria isolated were identified by the *Enterobacter* System gallery. Sensitivity tests were performed using the standard antibiotic susceptibility test by diffusion on Mueller Hinton medium. At the end of the analysis, 51 *Enterobacteriaceae* were isolated and identified. They included: 8 (15.68%) *Escherichia coli*, 8 (15.68%) *Salmonella spp.*, 8 (15.68%) *Shigella spp.*, 8 (15.68%) *Klebsiella spp.*, 5 (9.80%) *Enterobacter aerogenes*, 8 (15.68%) *Enterobacter cloacae*, 3 (5.90%) *Arizona spp.*, 3 (5.90%) *Proteus spp.* The results obtained show that the bacteria tested showed total resistance to the following antibiotics: amoxicillin, amoxicillin + clavulanic acid, cloxacillin and nalidixic acid. On the other hand, imipenem, cefuroxime, cefotaxime, ceftriazone and kanamycin were the most active antibiotics with low levels of resistance. The low resistance rates observed for imipenem, cefotaxime, cefuroxime and ceftriazone show that these antibiotics can be used for the treatment of infections caused by household wastewater bacteria.

Keywords

Antibiotic Therapy, *Enterobacteriaceae*, Sewage, Households

1. Introduction

After half a century of antibiotic use, the emergence and spread of bacterial re-

sistance is a critical public health issue [1]. The widespread use of broad-spectrum antibiotics in human medicine to treat infections without diagnosis of the specific pathogen involved and in animal husbandry is considered a major factor in bacterial resistance to antimicrobials [2]. In some regions, poor hygienic conditions may be the main cause [3]. Many of the diseases that affect the world's population are linked in part to inadequate domestic and industrial wastewater disposal. Without treatment, wastewater is a growing danger to human health and the natural environment because of its load of toxic chemicals and pathogenic micro-organisms (bacteria, viruses, parasites...). They are therefore a permanent threat to human and animal health [4]. Among the bacteria, we can mention *Enterobacteria*, a group of bacteria whose natural habitat is the gastrointestinal tract of many warm-blooded animals, including humans. These bacteria represent commensal germs. However, poor hygienic conditions related to the traditional aspect of household sanitation as well as the lack of environmental sanitation can cause the spread of these bacteria in the community. Consequently, these bacteria can acquire virulence factors and become opportunistic pathogens, capable of causing health problems such as gastroenteritis and extra-intestinal infections (affecting the urinary tract, blood and central nervous system) encountered in hospital and community settings [5]. To remedy infections caused by these bacteria, beta-lactam antibiotics are commonly used as first-line molecules. Unfortunately today, many cases of resistance have been observed during the treatment of infections caused by community bacteria. Thus, the purpose of this work is to evaluate the microbiological profile and antibiotic resistance of *Enterobacteria* isolated from household wastewater for management of these bacteria.

2. Materials and Methods

2.1. Material

2.1.1. Biological Material

The biological material consisted of different strains of *Enterobacteriaceae* isolated from household wastewater collected from four districts of Brazzaville (Château d'eau, Diata, Mougali and Poto poto) located respectively in the districts: 1 Makélékélé, 3 Poto-poto and 4 Mougali.

2.1.2. Laboratory Equipment

It consisted of the culture media for isolation (Eosine Blue Methylene Agar, SS); for the antibiogram (MH), the *Enterobacter* System gallery and the antibiotic discs. The following antibiotics were tested: amoxicillin (AX., 25 µg), amoxicillin + clavulanic acid (AMC., 20/10µg), cloxacillin (CX., 5 µg), imipenem (IMP., 10 µg), cefalotin (CEF., 30 µg), cefalexidine (CXN., 30 µg), cefuroxime (CXM., 30 µg), cefotaxime (CTX., 30 µg), cefotaxime (CTX., 30 µg), ceftazidime (CAZ., 30 µg), ceftazidime (CAZ., 30 µg), kanamycin (KAN., 1 mg), nalidixic acid (NA., 30 µg).

2.2. Methods

2.2.1. Sampling

Eight sites were selected, with two sites per neighbourhood (**Table 1**). A total of four (4) samples were collected per site over a two-month period. A one-week interval was observed between samples. 100 mL of water from each site was collected with a Pyrex glass vial after flaming the opening and then sent to the laboratory in a cooler. These different sites are wells or collectors of water from household use. Once full, these collectors are emptied and the water is poured into the streets.

2.2.2. Isolation

The various water samples were seeded on selective agar media. The following media were used:

- Methylene Blue Eosin Agar (EMB) for the isolation of *Enterobacteriaceae*,
- SS Agar for the isolation of *Salmonella* and *Shigella* genus.

2.2.3. Strain Identification

An *Enterobacter* System gallery comprising several biochemical reactions was used for the identification of *Enterobacteriaceae*.

2.2.4. Antibiotic Resistance

Resistance testing was done using the standard Mueller Hinton diffusion susceptibility testing method [6] [7]. An isolated colony of pure culture is suspended in 5 mL of sterile physiological water which constitutes the inoculum. Previously cast Petri dishes containing Mueller Hinton medium were inoculated by flooding. Excess inoculum is discarded in the bleach and then the plates are inverted and placed in the oven for 15 minutes. The antibiotic discs are then placed on the inoculated agar and the plates are incubated at 37°C for 18 to 24 hours. After 18 to 24 hours of incubation, the diffusion diameters of the different antibiotics were measured and compared to the reference diameters of the Antibiogram Committee of the French Society of Microbiology [8].

2.2.5. Determination of Resistance Phenotypes

They have been determined by reading the antibiogram, which identifies the clinical types (sensitive, resistant and intermediate). The resistance phenotype is the group of antibiotics to which the strain is resistant.

3. Results

3.1. Isolation and Identification of Strains

A total of 51 strains of *Enterobacteriaceae* were isolated. After identification, the strains of *Enterobacteriaceae* were as shown in **Figure 1**.

3.2. Antibiotic Resistance

The different antibiotic resistance results are shown in **Table 2** and **Table 3**.

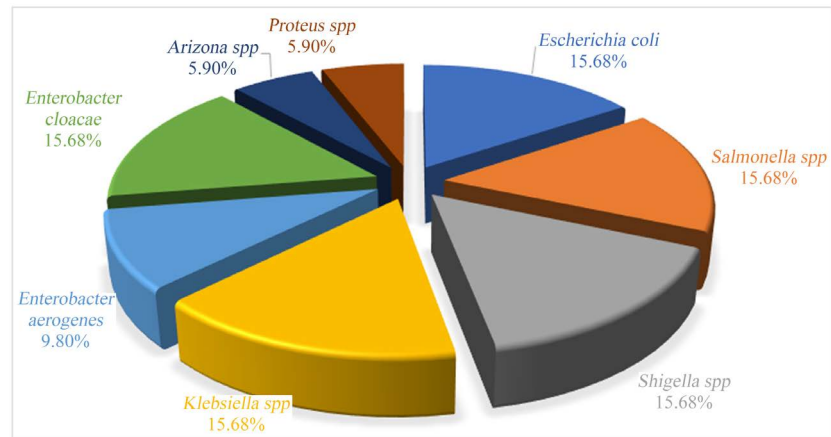


Figure 1. Distribution by identified species.

Table 1. Description of the different sampling sites.

Collection sites	Boroughs	Neighbourhoods	Addresses
Site 1	1	Diata	Fraternity Street No. 17
Site 2			Kimbenza Street No. 52
Site 3	1	Château d'eau	Massamba Bernard Street No. 10
Site 4			Saint Paul Street No. 13
Site 5	3	Poto-poto	Zandé Streek No. 16
Site 6			Bakoukouyas Street No. 14
Site 7	4	Moungali	Djambala Street No. 10
Site 8			Makotipoko Street No. 21

Table 2. Resistance rates of 8 *Escherichia coli*, *Salmonella spp.*, *Shigella spp.* and 3 *Arizona spp.*

ATB tested	<i>Escherichia coli</i> (n = 8)			<i>Salmonella spp.</i> (n = 8)			<i>Shigella spp.</i> (n = 8)			<i>Arizona spp.</i> (n = 3)			
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	
AX	8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	3 (100)	0	0	
AMC	8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	3 (100)	0	0	
CX	7 (87.5)	0	1 (12.5)	8 (100)	0	0	8 (100)	0	0	3 (100)	0	0	
IMP	1 (12.5)	0	7 (87.5)	4 (50)	1 (12.5)	3 (37.5)	2 (25)	0	6 (75)	1 (33.33)	1 (33.33)	1 (33.34)	
CEF	8 (100)	0	0	5 (62.5)	3 (37.5)	0	5 (62.5)	0	3 (37.5)	2 (66.67)	0	1 (33.33)	
CXN	8 (100)	0	0	5 (62.5)	3 (37.5)	0	5 (62.5)	0	3 (37.5)	2 (66.67)	0	1 (33.33)	
CAZ	8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	3 (100)	0	0	
CXM	2 (25)	0	6 (75)	8 (100)	0	0	8 (100)	0	0	2 (66.67)	0	1 (33.33)	
CTX	0	0	8 (100)	2 (25)	0	6 (75)	2 (25)	2 (25)	4 (50)	0	0	3 (100)	
CRO	0	0	8 (100)	2 (25)	0	6 (75)	2 (25)	0	6 (75)	0	0	3 (100)	
Global resistance	50 (62.5)	0	30 (37.5)	58 (72.5)	7 (8.75)	15 (18.75)	56 (72)	2 (2.5)	22 (25.5)	19 (63.33)	1 (3.33)	10 (33.34)	
Aminosides	KAN	0	0	8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	3 (100)
Global resistance		0	0	8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	3 (100)
Quinolones	NA	8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	3 (100)	0	0
Global resistance		8 (100)	0	0	8 (100)	0	0	8 (100)	0	0	3 (100)	0	0

R: Resistance; I: Intermediate; S: Sensitive; %: Percentage; n: Number; ATB: Antibiotics. Amoxicillin (AX), amoxicillin + Clavulanic Acid (AMC), cloxacillin (CX), imipenem (IMP), cefalotin (CEF), cefalexidine (CXN), cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), kanamycin (KAN), nalidixic acid (NA), cefalexidine (CXN), cefuroxime (CXM), cefotaxime (CTX), ceftriazone (CRO), ceftazidime (CAZ), kanamycin (KAN), nalidixic acid (NA).

Table 3. Resistance rates of 8 *Klebsiella spp.*, *Enterobacter aerogenes*, 8 *Enterobacter cloacae* and 3 *Proteus spp.*

ATB tested	<i>Klebsiella spp.</i> (n = 8)			<i>Enterobacter aerogenes</i> (n = 5)			<i>Enterobacter cloacae</i> (n = 8)			<i>Proteus spp.</i> (n = 3)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
AX	8 (100)	0	0	5 (100)	0	0	8 (100)	0	0	3 (100)	0	0
AMC	8 (100)	0	0	-	N t	N t	-	N t	N t	3 (100)	0	0
CX	8 (100)	0	0	5 (100)	0	0	8 (100)	0	0	3 (100)	0	0
IMP	0	0	8 (100)	0	0	5 (100)	1 (12.5)	0	7 (87.5)	0	0	3 (100)
CEF	1 (12.5)	0	7 (87.5)	-	N t	N t	-	N t	N t	0	0	3 (100)
CXN	4 (50)	0	4 (50)	-	N t	N t	-	N t	N t	1 (33.33)	0	2 (66.67)
CAZ	7 (87.5)	0	1 (12.5)	0	0	5 (100)	0	0	8 (100)	3 (100)	0	0
CXM	1 (12.5)	2 (25)	5 (62.5)	0	0	5 (100)	4 (50)	2 (25)	2 (25)	3 (100)	0	0
CTX	2 (25)	0	6 (75)	0	0	5 (100)	2 (25)	0	6 (75)	0	0	3 (100)
CRO	0	0	8 (100)	0	0	5 (100)	7 (87.5)	0	1 (12.5)	0	0	3 (100)
Global resistance	39 (48.75)	2 (2.5)	39 (48.75)	10 (28.58)	0	25 (71.42)	30 (53.57)	2 (3.57)	24 (42.86)	16 (53.33)	0	14 (46.67)
Aminosides	KAN	0	8 (100)	0	0	5 (100)	0	0	8 (100)	0	0	3 (100)
Global resistance		0	8 (100)	0	0	5 (100)	0	0	8 (100)	0	0	3 (100)
Quinolones	NA	8 (100)	0	5 (100)	0	0	8 (100)	0	0	3 (100)	0	0
Global resistance		8 (100)	0	5 (100)	0	0	8 (100)	0	0	3 (100)	0	0

R: Resistance; I: Intermediate; S: Sensitive; %: Percentage; n: Number; ATB: Antibiotics; -: Natural resistance; N t: Not tested. Amoxicillin (AX), amoxicillin + Clavulanic Acid (AMC), cloxacillin (CX), imipenem (IMP), cefalotin (CEF), cefalexidine (CXN), cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), kanamycin (KAN), nalidixic acid (NA), cefalexidine (CXN), cefuroxime (CXM), cefotaxime (CTX), ceftriazone (CRO), ceftazidime (CAZ), kanamycin (KAN), nalidixic acid (NA).

Figure 2 shows the distribution of resistance among the different strains of *Enterobacteriaceae*. This figure shows that *Enterobacteriaceae* have shown high levels of resistance to ampicillin, amoxicillin, amoxicillin + clavulanic acid, cloxacillin, ceftazidime and nalidixic acid. However, imipenem, cefuroxime, cefotaxime, ceftriazone and kanamycin were more active.

All strains of *Enterobacteriaceae* have been tested with three families of antibiotics. Of all the families tested, the aminoglycosides (Kanamycin) retained their activity on all strains of *Enterobacteriaceae* with zero resistance. On the other hand, total resistance was observed for the quinolone family (nalidixic acid). The frequencies ranged from 28.58% to 72.5% respectively for *Enterobacter aerogenes* and *Salmonella spp.* to beta-lactam resistance (**Figure 3**).

3.3. Resistance Phenotype

The phenotypic resistance patterns observed in *Enterobacteriaceae* were as follows: 5 strains presented the AX AMC CX CEF CXN CAZ CXM CTX CRO phenotype (15.6%); 4 strains presented the AX AMC CX IMP CEF CXN CAZ phenotype (12.5%); 19 strains presented the AX AMC CX CEF CAZ phenotype (59.37%); 3 strains presented the AX AMC CX CEF CXN CAZ CTX phenotype

(9.38%) and 1 strain presented the AX AMC CX CEF CAZ CXM CRO (3.15%). The presence of these resistance phenotypes in *Enterobacteriaceae* underscores the inactivation of beta-lactam antibiotics by extended-spectrum beta-lactamases (Figure 4).

4. Discussion

This study assessed the antibiotic resistance of *Enterobacteriaceae* isolated from household wastewater in four neighbourhoods of the city of Brazzaville to antibiotics used in the treatment of *Enterobacteriaceae* infections.

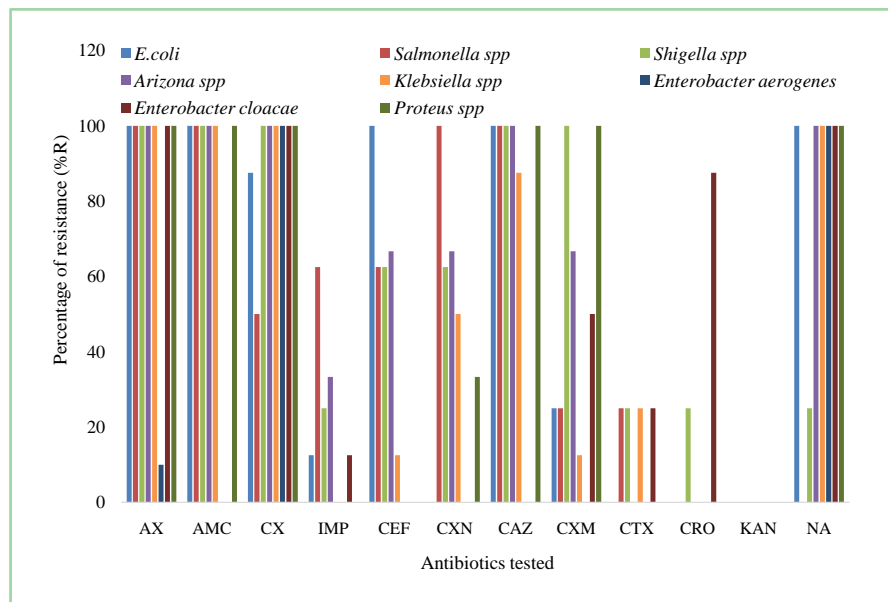


Figure 2. *Enterobacteriaceae* resistance profile.

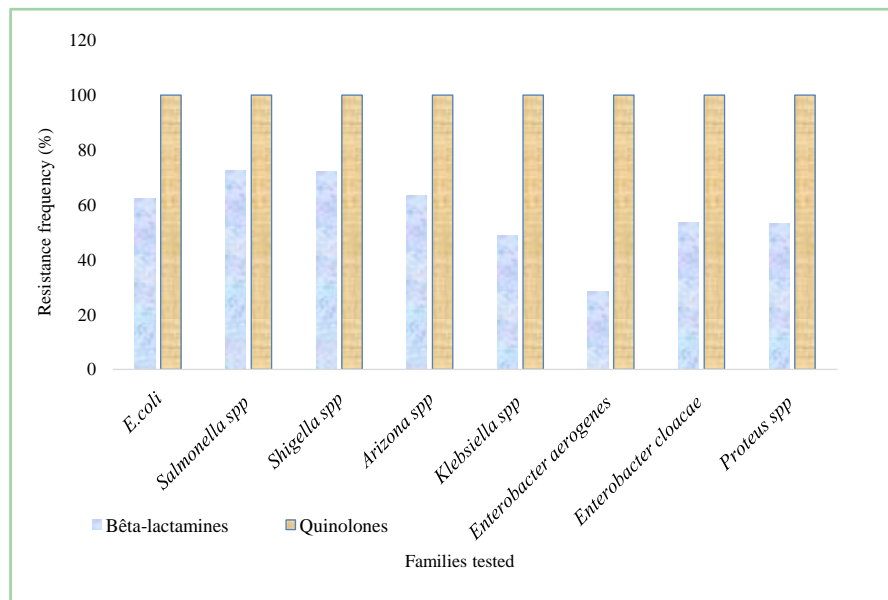


Figure 3. Frequency of resistance by family as a function of species.

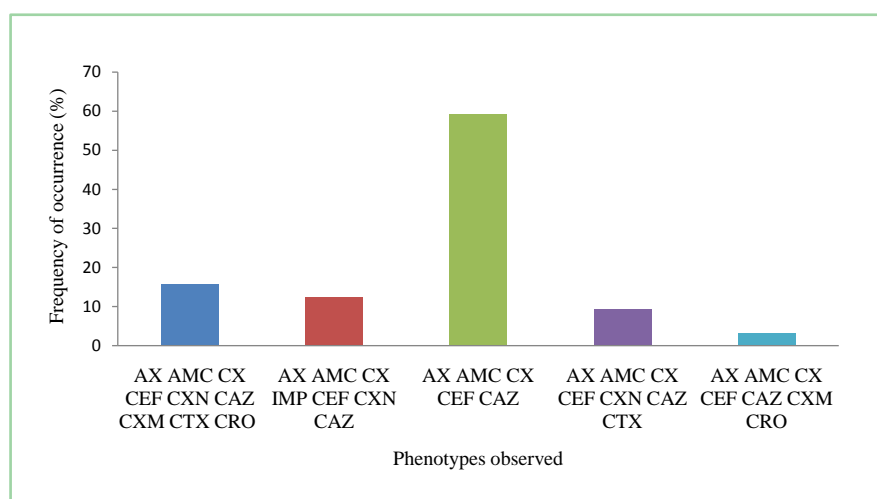


Figure 4. Resistance phenotypes observed in *Enterobacteriaceae*.

This study revealed resistance rates in *E. coli* of 100% for amoxicillin/clavulanic acid and cefalotin. Lower rates were reported in a similar study conducted by Servais *et al.* [9] in Belgium on *E. coli* strains isolated from the waters of the Seine basin. In fact, 662 *E. coli* strains showed resistance rates of 18.6%, 17.4% to amoxicillin/clavulanic acid and cefalotin, respectively [9]. In Morocco, Tagajdid *et al.* [10] working on *E. coli* strains isolated from consulting patients in the laboratory of the Cheikh Zayd University Hospital in Rabat reported a 10% resistance rate to amoxicillin/clavulanic acid. In contrast, Mpelle *et al.* [11] reported similar resistance rates (100%) for amoxicillin, amoxicillin + clavulanic acid, and cefalotin each on *E. coli* strains isolated at the Brazzaville Hospital and University Centre. We reported 100% resistance rates in *E. coli* to amoxicillin, amoxicillin + clavulanic acid, cefalotin, ceftazidime and nalidixic acid. Lower percentages of resistance were reported by Passerat *et al.* [12] 25%, 15%, 20%, 0% and 25% respectively; Moyen *et al.* [13] reported 100%, 87%, 87% for amoxicillin, amoxicillin acid/clavulanic acid, cefalotin and ceftazidime. In this study, the rates of resistance to ceftriazone and imipenem in *E. coli* strains were 0% and 12.5%, respectively. These frequencies are in disagreement with those reported by Mpelle *et al.* [11] on *E. coli*, whose respective rates are 79.07% and 0%, and by Moyen *et al.* [13] 73.9% and 4.3%. Oubrim *et al.* [14] reported a zero resistance rate for the same antibiotics tested.

Our results on *Enterobacter cloacae* and *Enterobacter aerogenes* (100%) for amoxicillin are comparative to those found by Oubrim *et al.* [14] (100%). In this study, resistance rates on *Proteus spp.* were for amoxicillin (100%), cefalotin (0%), cefuroxime (100%), cefuroxime (100%), ceftriaxone (0%), cefotaxime (0%), ceftazidime (100%), kanamycin (0%), nalidixic acid (100%). Different rates of resistance were observed by Souna [15] for amoxicillin (70.4%), amoxicillin/clavulanic acid (55.5%), cefalotin (51.8%), cefuroxime (63%), ceftaxitin (29.6%), ceftriaxone (25.9%), cefotaxime (33.3%), ceftazidime (37%), kanamycin (33.3%), for nalidixic acid (33.3%) and imipenem (11.1%). Moyen *et al.* [13] reported resistance rates

of 91%; 86.5%; 41%; 91%; 45.5%; 22.7% respectively for amoxicillin, cefalotin, cefuroxime, cefuroxime, cefttriazone, cefotaxime, ceftazidime. Our results on *Enterobacter cloacae* are compared to those found by Souna [15], with respect to amoxicillin (100%), amoxicillin/clavulanic acid (100%) whose respective percentages are 100% each. The frequency of resistance in *Enterobacteriaceae* (0%) for kanamycin differs from that found by Souna [15] for kanamycin (17.2%). The result on *Shigella spp.* to nalidixic acid (100%) is compared to that found by Kasolo [16] for 18 strains tested with nalidixic acid (100%). During our study, our results on nalidixic acid for *E. coli* (100%) and *Klebsiella spp.* (100%) strains differed from those found by Efuntoye *et al.* [17] with the respective percentages of 25.7% and 37%. The high resistance rates could be explained by either diffusion of the clinical strains in the city or transfer of resistance genes from the clinical strains to the community strains. The phenotypes that emerged for amoxicillin and amoxicillin/clavulanic acid could be due to the production of a penicillin-binding protein [18] or to beta-lactamases (cephalosporinases) [19].

In this study, the resistance rates of *Escherichia coli*, *Proteus spp* and *Klebsiella spp.* strains to amoxicillin/clavulanic acid, cefttriazone and nalidixic acid were 100%, 0% and 100% respectively for each species. In France, Pulcini *et al.* [20] working on strains of *Escherichia coli*, *Proteus spp.* and *Klebsiella spp.* isolated from people living in retirement homes reported resistance rates for amoxicillin/clavulanic acid, cefttriazone and nalidixic acid (18.5%, 5.5%, 19.5%), (13.5%, 0.2%, 25.5%) and (12.2%, 4.1%, 11.4%) respectively.

Microorganisms present in wastewater or surface water develop long-term antibiotic resistance due to exposure to low concentrations of antibiotics (ng /l to mg /l) [21]. Strain resistance to some antibiotics (beta-lactam antibiotics, some cephalosporins) can be explained by the fact that these antibiotics are used in human and veterinary medicine with no limits on their use (they are most commonly prescribed in ambulatory practice) [9] and by the frequency and extent of wastewater flow from hospitals to the community.

5. Conclusion

This study allowed us to determine the antibiotic resistance rates of *Enterobacteria* isolated from household wastewater and to establish the corresponding phenotypes. The results show that the *Enterobacteria* showed high rates of resistance to amoxicillin, amoxicillin + clavulanic acid, cloxacillin, ceftazidime and nalidixic acid. Imipenem, cefuroxime, cefotaxime, ceftriaxone and kanamycin were more active. Out of ten (10) beta-lactam antibiotics tested, *Enterobacteriaceae* were resistant to nine (9) antibiotics with five (5) different profiles, the majority of which were attributed to resistance to six (6) antibiotics (AX AMC CX CEF CAZ). The observed phenotypes involve both penicillin and cephalosporin inhibition. These are the predominant penicillinase and cephalosporinase activities. However, some molecules such as imipenem, cefuroxime, cefotaxime, ceftriaxone and kanamycin can be used for the treatment of infections due to these

bacteria in household wastewater. To overcome the phenomenon of resistance, these molecules should not be used as monotherapy. Therefore, household wastewater should not be discharged directly into the environment without prior treatment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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