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Survey of Multi-Resistant Bacteria in Waste Water from the Yopougon Hospital and University Center (CHU), The Abattoir of Port-Bouët and the Lagoon Bays of the City of Abidjan (Côte d'Ivoire)

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A B S T R A C T

Antibiotic resistance has become a global concern due to their excessive use in human and veterinary medicine. The purpose of the study was to determine the level of antibiotic resistance in multi-resistant gram-negative enterobacteria and bacilli isolated from the effluents of the Port-bouët abattoir, the Yopougon Hospital and University Center, and the lagoon bays of the city of Abidjan. It was conducted over the period of January to December 2019. A total of 192 wastewater samples were collected and analyzed at the Microbiology laboratory of ivorian antipollution Center. The antibiogram was performed at the Institut Pasteur of Côte d'Ivoire. The bacteriological analysis allowed the isolation of 34 species of enterobacteria and 19 non-fermentative gram-negative bacilli with a respective predominance of *Klebsiella pneumoniae* (41.2%) and *Aeromonas hydrophila* (42.1%). The study also revealed high resistance rates of 50-100% to beta-lactams in enterobacteria and gram-negative bacilli with almost no resistance to imipenem. High resistance has also been observed with some quinolone antibiotics, aminoglycosides, phenicol, fosfomycin and sulfamethoxazole. The presence of multi-resistant bacteria in these effluents represents a real risk for public health.

Introduction

Antibiotic resistance has become a major concern, leading to a real awareness both nationally and internationally, especially since in recent years, the development of new antibiotic molecules has become rare (Cizman and Plankar, 2018).

The overuse of antibiotics, both in human and veterinary medicine, or as a growth factor in animal husbandry, has led to the emergence and spread of antibiotic-resistant bacteria. The overuse of antibiotics, both in human and veterinary medicine, or as a growth promoter in animal husbandry, has led to the emergence and spread of bacteria that are multi-resistant to antibiotics (Meyer *et al.,* 2013; Beyene and Tesega, 2014).

In addition to the hospital environment and animal husbandry, multi-resistant bacteria have now spread in the environment, and their detection in wastewater (hospital and municipal effluents), at wastewater treatment plants, in surface or ground water, and in soil is now established (Baquero *et al.,* 2008; Rizzo *et al.,* 2013).

Therefore, hospital and municipal effluents have been identified as potential reservoirs of multi-resistant bacteria in general and enterobacteria in particular (Tennstedt *et al.,* 2003).

Indeed, the strong selection pressure represented by the release of large quantities of antibiotics into nature and the pollution of water by commensal enterobacteria from humans and animals are strong arguments for thinking that the environment is a reservoir of multi-resistant bacteria (Kummerer, 2004).

The problem of antimicrobial resistance increasingly tends to be seen as a global environmental issue. The commensal intestinal flora of humans, animals and the bacterial flora of hospital and municipal effluents are now considered as a potential reservoir of antibiotic-resistant bacteria and/or resistance genes, potentially transmissible to humans (Goni-Urriza *et al.,* 2000 ; Bonnedahl *et al.,* 2009). A public health problem then arises because humans, in the various environments in which they live, may be exposed to these multi-resistant bacteria, particularly through their activities with water (watering market gardening crops, swimming, etc.). On the other hand, their presence in the environment could lead to the transfer of resistance genes to environmental bacteria, thus causing an even greater spread of these antibiotic resistances.

The objective of this study was to contribute to the monitoring of multi-resistant gram-negative bacteria in the environment through the effluents of the municipal abattoir of Port-Bouët, the hospital and university center (CHU) of Yopougon and the lagoon bays of the city of Abidjan. Specifically, the aim was to (i) isolate resistant bacteria from the effluents of the Port-Bouët slaughterhouse, the Yopougon hospital and university hospital center (CHU) and the waters of the lagoon bays, (ii) study the resistance profiles of the multi-resistant strains isolated.

Materials and Methods

This was a study of the multiresistance of strains of gram-negative bacteria isolated from the effluents of the municipal abattoir of Port-Bouët, the CHU of Yopougon and the lagoon bays of the city of Abidjan.

The geographical coordinates of the different sampling sites (stations) in the District of Abidjan are recorded in Table 1. Each site was subject to water sampling at 0.5 meters from the surface of the water body. Thus, from January to December 2019, two campaigns of 8 waters samples were taken monthly, i.e. 16 samples per month.

The geographical coordinates of the different sampling sites (stations) in the District of Abidjan are recorded in Table I. Each site was subject to water sampling at 0.5 meters from the surface of the water body. Thus, from January to December 2019, two campaigns of

8 waters samples were taken monthly, i.e. 16 samples per month.

A total of 192 waters samples were collected for this study and placed in a cooler containing cold accumulators and transported to the laboratory for bacteriological analysis.

The isolation and identification of multi-resistant gram-negative bacteria were carried out at the microbiology laboratory of the Ivorian Anti-Pollution Center. Decimal dilutions carried out on the different water samples were used to isolate multi-resistant gram-negative bacilli on media made selective by the addition of antibiotic powder.

Drigalski agars with 4 mg/L of ceftazidime were used to isolate multi-resistant enterobacteria and non-fermenting gram-negative bacteria.

Cetrimide agars with 8 mg/L ceftazidime and 8 mg/L imipenem have been used for the detection and identification of multi-resistant bacteria of the genus *Pseudomonas*. The inoculation of the different agars with antibiotic was performed by spreading 0.1 mL of the different dilutions.

All Petri dishes were incubated at 37°C for 24 hours. The identification of multi-resistant Gram-negative bacilli was carried out using the Api20E gallery (Biomérieux, France) after the fresh state, the Gram stain and the catalase test.

All antibiotic susceptibility tests were performed at the laboratory of the national antibiotic reference center of the Institut Pasteur of Côte d'Ivoire. Antimicrobial susceptibility of β extended-spectrum enterobacteria β-lactamase isolates was determined by the Bauer-Kirby disc diffusion test using antibiotic discs (Bio-Rad, France) (Bauer *et al.,* 1996).

Results and Discussion

Microbiological analysis of the water samples analyzed revealed 34 species of Enterobacteriaceae with a predominance of *Klebsiella pneumoniae* species, i.e. 14 isolated species (41.2%). On the other hand, 19 non-fermentative gram-negative bacilli were identified with a predominance observed with the 8 *Aeromonas hydrophila* species, i.e., 42.1% (Tables 2 and 3).

Sensitivity tests performed on isolated bacteria revealed high rates of resistance to the same families of antibiotics as ESBL enterobacteria in non-fermentative gram-negative bacilli. Most of the isolated Enterobacteriaceae were resistant to 5 families of antibiotics including the beta-lactam, quinolone, aminoglycoside, phenicol and other families (Fosfomycin, Trimethoprim/sulfamethoxazole). Concerning non-fermentative gram-negative bacilli, resistance has been observed with the families of beta-lactam antibiotics, quinolones, aminoglycosides.

The omnipresence of multi-resistant bacteria in the environment has been demonstrated worldwide (Blaak *et al.,* 2015 ; Zhang *et al.,* 2016). Multiresistant gram-negative bacteria, particularly BLSE enterobacteria and multiresistant non-fermentative gram-negative bacilli, have been detected in various samples of effluents from the Port-Bouët slaughterhouse, the Yopougon university hospital and the waters of the lagoon bays of Abidjan during various sampling campaigns. In Côte d'Ivoire, the hospital effluent evacuation network consists either of a pipe leading directly to the receiving environment (Ebrié lagoon), as is the case in some hospitals and universities, or it is connected to the municipal wastewater network.

The connection of the hospital effluent evacuation network to the municipal

wastewater evacuation network could justify, in part, the presence of multi-resistant bacteria in the waters of the lagoon bays.

In the study on the isolation of broad-spectrum beta-lactamase-producing Enterobacteriaceae isolated from lagoon berries, *Klebsiella pneumoniae* and *Escherichia coli* were the most isolated bacteria. The predominance of these species had already been highlighted during a previous study carried out by (Ouattara *et al.,* 2014) on broad-spectrum beta-lactamase-producing enterobacteria of various origins (human, animal and environmental) in Abidjan, Côte d'Ivoire. The presence of ESBLs in surface waters has been frequently demonstrated worldwide, leading to the conclusion that if bacteria in the water are able to host ESBL genes, ESBLs will be present in the population (Zurfluh *et al.,* 2013; Blaak *et al.,* 2015; Zhang *et al.,* 2016; Zarfel *et al.,* 2017). The spread of ESBLs is facilitated by the location of most ESBL genes on mobile genetic elements that allow the transmission of resistance genes to strains and species better adapted to the surface water environment. As a result, environmental bacteria can acquire resistance genes from, for example, clinically derived strains (Zurfluh *et al.,* 2013; Blaak *et al.,* 2015; Zhang *et al.,* 2016; Zarfel *et al.,* 2017).

All these findings confirm that ESBL enterobacteria, which were originally described as hospital bacteria, have spread widely in the environment. Among the multi-resistant gram-negative bacilli, the *Aeromonas hydrophila* and *Pseudomonas aeruginosa* species were the most isolated. These multi-resistant bacteria are heavily implicated in hospital infections. The presence of these species seems to be again related to the discharge of hospital waste water directly into the receptacle that is the Ebrié lagoon of Abidjan. In addition, *Pseudomonas aeruginosa*, a hydrophilic species for which hospital wastewater may be an ideal habitat (Tumeo *et al.,* 2008). Their presence in hospital effluents has been demonstrated by several authors (Schwartz *et al.,* 2003 ; Tumeo *et al.,* 2008).

Overall, strains of Enterobacteriaceae have shown a high level of antibiotic resistance. This could be explained by the strong selection pressure due to the often abusive and uncontrolled use of antibiotics in both human and veterinary medicine and the presence of unmetabolized antibiotic residues in effluents. In addition, the production of ESBLs is often associated with resistance to several other antibiotics with the same genetic carriers as ESBLs (Paterson, 2006).

These are the genes for resistance to aminoglycosides (Gentamycin; Amikacin), acetylase AAC6'-Ib-cr, cotrimoxazole (SXT), and the plasmid gene for resistance to quinolones (Nalidixic acid; Ciprofloxacin), qnr (Martínez-Martínez, 2006). All isolated strains produce broad-spectrum beta-lactamase regardless of strain origin. All isolated BLSE strains have a very high level of resistance to C3G and C4G. According to Winokur (2001), EBLSEs, due to their genomic characteristics, are usually resistant to C3G and Aztreonam, but may be sensitive to C4G and carbapenems, beta-lactam antibiotics depending on the resistance genes they host.

In addition to the antibiotics used to characterize the EBLSE phenotype, high resistance of the strains was observed to Amoxicillin + clavulanic acid. A level of resistance of 50% to Cefoxitin was also observed. These levels could be explained by the simultaneous synthesis of AmpC-type cephalosporinases, which confers EBLSE resistance to clavulanic acid.

The resistance of EBLSEs to C3G reflects the

extensive use of C3G and Aztreonam in human therapeutics. Indeed, several antibiotics including Carbapenems, Cefoxitin, C3G and clavulanic acid are inducers of beta-lactamases. C3Gs present a very high selection risk because they eliminate wild (susceptible) bacteria and bring out those that hyperproduce high-level cephalosporinase. The ESBL enterobacteria strains isolated were also resistant to fosfomycin, an antibiotic used in human medicine for the treatment of ESBL enterobacteria infections (Winokur, 2001).

Table.1 Geographic coordinates of sampling stations

Stations	GPS coordinates
Yopougon Béago	05°18'316N ; 04°03'917W
Attécoubé Boribana	05°19'691N ; 04°01'969W
Port bouët Zimbaboué	05°16'752N ; 04°00'098W
Port bouet Abattoir	05°15'492N ; 03°58'132W
Koumassi digue	05°16'457N 03°55'801W
Marcory Biafra	05°19'677N ; 04°00'833W
Cocody M'badon	05°19'955N ; 03°55'737W
Yopougon Centre Hospitalier et Universitaire	05°21'207N 04°05'049W

Table.2 Frequency of Isolated Enterobacteriaceae Species

Enterobacteriaceae	Numbers	Percentage (%)
Klebsiella pneumoniae	14	41,2
Escherichia coli	7	20,6
Enterobacter aerogenes	4	11,8
Citrobacter freundii	3	8,8
Serratia liquefaciens	3	8,8
Enterobacter cloacae	2	5,9
Raoutella ornithinolytica	1	2,9
Total	**34**	**100**

Table.3 Frequency of Isolated Gram-Negative Bacilli

Non-enterobacteria	Numbers	Percentage (%)
Aeromonas hydrophila	08	42,1
Pseudomonas aeruginosa	04	21,1
Vibrio fluvialis	04	21,1
Pseudomonas fluorescens	02	10,5
Burkholderia cepacia	01	5,3
Total	**19**	**100**

Table.4 Antibiotic Resistance rates of Enterobacteriaceae Isolated

	Number of resistant strains (I+R%)						
	K. pneumoniae (N=14)	*E. coli* (N= 7)	*E. aerogenes* (N=4)	*C. freundii* (N= 3)	*S. liquefaciens* (N= 3)	*E. cloacae* (N=1)	*R.ornithinolytica* (N=1)
Bêta-lactamines							
Amoxicilline+acide clavulanique*	14 (100)	7 (100)	4 (100)	3(100)	3 (100)	2 (100)	1(100)
Ceftazidime*	14 (100)	7 (100)	4 (100)	3 (100)	3 (100)	2 (100)	1(100)
Ceftriaxone *	14 (100)	7 (100)	4 (100)	3 (100)	3 (100)	2 (100)	1(100)
Cefepime*	14 (100)	7 (100)	4 (100)	3 (100)	3 (100)	2 (100)	1(100)
Cefotaxime*	14 (100)	7 (100)	4 (100)	3 (100)	3 (100)	2 (100)	1(100)
Cefoxitine	7 (50)	0 (0)	2 (50)	3 (100)	3 (100)	2 (100)	1(100)
Imipénème	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Quinolones							
Acide nalidixique	9 (64,3)	7 (100)	2 (50)	3 (100)	3 (100)	2 (100)	1(100)
Ciprofloxacine	9 (64,3)	4 (50)	2 (50)	3 (100)	3 (100)	2 (100)	1(100)
Levofloxacine	9 (64,3)	4 (50)	2 (50)	3(100)	3 (100)	2 (100)	1(100)
Aminosides							
Gentamicine	4 (35,7)	0 (0)	2 (50)	3 (100)	3 (100)	0 (0)	1(100)
Tobramycine		0 (0)	2 (50)	3 (100)	3 (100)	0 (0)	1(100)
Amikacine	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	0 (0)	0(0)
Phénicolés							
Chloramphénicol	4 (35,7)	0 (0)	4 (100)	3 (100)	0 (0)	2 (100)	0 (0)
Autres familles							
Fosfomycine	4 (35,7)	0 (0)	2 (50)	0 (0)	0 (0)	2 (100)	0 (0)
Trimethoprime/sulfamethoxazole	9 (64,3)	7 (100)	2 (50)	3 (100)	3 (100)	2 (100)	1(100)

Table.5 Antibiotic resistance rates of isolated gram-negative non-fermentative bacilli

Number of resistant strains (I+R%)					
Antibiotics	*Aeromonas hydrophila* (N=8)	*Pseudomanas aeruginosa* (N= 4)	*Vibrio fluvialis* (N= 4)	*Pseudomanas fluorescens* (N=2)	*Burkholderia cepacia* (N= 1)
Bêta-lactamines					
Ticarcilline+acide clavulanique*	8 (100)	4 (100)	4 (100)	2 (100)	1 (100)
Ceftazidime*	6 (75)	4 (100)	4 (100)	2 (100)	1 (100)
Ceftriaxone *	6 (75)	4 (100)	4 (100)	2 (100)	1 (100)
Cefepime*	4 (50)	2 (50)	4 (100)	2 (100)	1 (100)
Cefotaxime*	6 (75)	4 (100)	4 (100)	2 (100)	1 (100)
Imipénème	8 (100)	0 (0)	4 (100)	2 (100)	1 (100)
Quinolones					
Ciprofloxacine	4 (50)	2 (50)	4 (100)	0 (0)	1 (100)
Levofloxacine	4 (50)	2 (50)	4 (100)	0 (0)	0 (0)
Aminosides					
Tobramycine	2 (25)	2 (50)	4 (100)	0 (0)	1 (100)
Gentamicine	6 (75)	0 (0)	0 (0)	0(0)	0 (0)
Netimicine	6 (75)	0 (0)	0 (0)	0 (0)	0 (0)
Amikacine	6 (75)	0 (0)	0 (0)	0 (0)	0 (0)

The observation of high levels of resistance to the same antibiotics as ESBL in non-fermentative Gram-negative bacilli could also be explained by the presence of numerous resistance genes on large plasmids that could be hosted by bacteria. Indeed, many resistance genes have migrated from bacteria naturally carrying these genes to environmental bacteria such as Pseudomonas. In addition, many phenomena of exchange of resistance genes such as conjugation, transformation allow bacteria in the environment to acquire many resistance genes in order to ensure their survival. The very high rate of resistance observed to carbapenems in non-fermentative Gram-negative bacilli is thought to be related to the presence of certain carbapenem resistance genes such as IMP, VIM and SIM genes.

The present study highlighted the presence of multi-resistant bacteria (BLSE enterobacteria and non-fermenting gram bacilli) in wastewater and surface water (lagoon water). Particularly high rates of resistance to often major antibiotics were observed and in general, isolated ESBL enterobacteria are resistant not only to several families of antibiotics but also to several antibiotics of the same family. This observation once again raises the issue of the impact of the discharge of certain untreated effluents such as hospital and municipal effluents directly into the lagoon environment. The presence of these multi-resistant bacteria would represent a major public health problem.

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