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Dissemination of Resistance Integrons and Genes Coding for Blse and Cabapenemases in the Urban Drainage Network in Cote d'Ivoire

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Abstract

Antibiotic resistance has become a major threat to human health worldwide. Environment, particularly the water environment, has long been overlooked as a player in the antibiotic resistance cycle, although its role remains unclear. These can provide an ideal setting for the acquisition and dissemination of antibiotic resistance, as they are frequently affected by anthropogenic activities. The objective of this study was to establish a diffusion map of resistance integrons used as genetic markers of resistance associated with antibiotic resistance conferring genes (ARGs). Total DNA extracts from non-cultivable bacterial communities were used for the analyses. These communities were obtained from wastewater samples from 14 sites upstream and downstream of drainage channels or effluents in the cities of Abidjan, Bouaké, and Yamoussoukro. The results obtained correspond to the number of positives among the treated samples (n = 39). Among the genetic markers of dissemination, class 1 integrons were the most evident in 94.8% of samples in Abidjan (93.3%), Bouaké (100%) and Yamoussoukro (91.6%). Class 2 integrons and class 3 integrons were found respectively in 41% and 51% of all samples. Genes coding for β-lactamases and blaTEM was identified in almost all samples at a rate of 97.4%. A co-presence of the three genes *blaTEM*, *blaSHV* and *blaCTX-M* is also remarkable in the sites of the city of Yamoussoukro. Among the genes coding for carbapenemases, only *blaKPC* 17.94%, *blaNDM* 30.76% and *blaOXA*48 38.46% were detected in the samples.

Keywords

Antibiotic Resistance, Wastewater, Resistance Integrons (RIs), Resistance

Genes

1. Introduction

Antibiotic resistance is a major global public health problem due to the use and misuse of antibiotics, which promotes the emergence and spread of resistant bacteria [1].

Since the first alert on antibiotic resistance, this phenomenon has dramatically increased. In 2019, according to the Lancet, the most comprehensive estimate to date of the global impact of antimicrobial resistance (AMR), 1.2 million people and potentially millions more have died from antibiotic-resistant bacterial infections [2]. Treating these infections increases healthcare costs as these therapies require longer hospital stays and more expensive drugs. To address this growing problem, it is necessary to understand the ecology of antibiotic resistance, including its origins, evolution, selection, and dissemination [3]. Although antibiotic resistance has been researched in clinically relevant human pathogens, environmental reservoirs of antibiotic resistance determinants and their contribution to resistance in clinical settings have only been considered in the last decade [4].

It is only very recently that the field of investigation has broadened to include the environment as a matrix for the evolution of antibiotic resistance. According to the "One Health" global control strategy, the study of antibiotic resistance in the environment could contribute to a better understanding of human epidemiology. This approach, which uses the environment as a sentinel of human health, is emerging especially in the face of the multiple anthropogenic pressures exerted on the different environmental compartments, which always require multidimensional studies [5]. The areas closest to humans can be used as an indicator or monitoring tool for antibiotic resistance with a view to implementing control policies adapted to the ecology of multi-resistant bacteria [6]. Antibiotic resistance genes (ARGs) have been shown to have environmental origins, but the introduction and accumulation of antimicrobials in the environment facilitate their spread [7]. As a result, ARGs can be found in almost any environment and are currently considered emerging pollutants [8]. Therefore, identifying resistance genes, their distribution in the environment and how anthropogenic inputs affect their spread will help to establish strategies to combat antibiotic resistance. The localisation of ARGs on genetic elements that can be mobilised, such as transposons, integrons and plasmids, facilitates the transfer of resistance to other organisms of the same or different species [6].

Urban cities in African countries combine the conditions to be "hot spots" for the emergence and spread of antibiotic resistance and are, therefore, areas that deserve further study [9]. Their many economic assets have contributed to the attraction of national and African populations in search of employment and social welfare. The result is a strong demographic pressure that has really caught

decision-makers and urban planners in charge of managing urban centres unaware. The high cost of living pushes the most disadvantaged populations to live in precarious neighbourhoods characterised by almost non-existent drinking water supply structures, sanitation networks and adequate waste collection systems [10]. Among the sanitation structures, large open drains have been assigned to drain rainwater to natural water bodies. However, in addition to rainwater, these structures carry effluents of various kinds and households that are not connected to the sewerage system discharge their domestic wastewater into them without any prior treatment, thus contributing to the biological pollution of the natural water receiving environments [11].

Although studies on antibiotic resistance have focused on culturable bacteria and/or indicator organisms in treated wastewater, most bacteria in the environment cannot be cultured under conventional conditions. Therefore, there is little information on how wastewater effluents can affect bacterial communities and impact the prevalence of genetic markers and resistance genes in the environment [12]. However, due to the adaptive advantage they provide, long-term exposure to low amounts of antibiotics may increase the genetic stabilization of these resistance determinants [13]. Due to the overuse and misuse of antimicrobials, selective pressure is exerted on bacteria to significantly increase antimicrobial resistance, resulting in a loss of efficacy of antimicrobial treatments. The β-lactams are by far the most widely consumed antibiotics in the world [14]. This class of antibiotics includes penicillins, cephalosporins, monobactams and carbapenems. Carbapenems, such as imipenem or meropenem, have the broadest spectrum of activity and bactericidal activity against many gram-negative and gram-positive bacteria [15] [16]. Their particular chemical structure gives them protection against most β-lactamases produced by bacteria, including cephalosporinases or extended-spectrum β-lactamases (ESBLs) [16] [17]. Therefore, carbapenems are considered antibiotics of last resort and are used to treat bacterial infections when all other treatment options fail [17]. Bacterial resistance to carbapenems is therefore an important public health problem. Among the determinants of carbapenem resistance, carbapenemase enzymes are the most clinically relevant and worrisome because: 1) these enzymes hydrolyse almost all β-lactams, including carbapenems, which considerably limits therapeutic options; and 2) the clinically relevant carbapenemase enzymes are the most important for the treatment of bacterial infections.

With a view to understanding the mechanisms of antibiotic resistance and therefore, to some extent, its control, the detection of resistance integrons (RIs) associated with different resistance genes can be crucial. The integrons involved in antibiotic resistance are grouped into several classes depending on the nature of their integrase. Integrons can be divided into two major groups divided into 4 classes: resistance integrons (classes 1, 2 and 3) are the best characterised and recognised as being involved in the dissemination of antibiotic resistance, and super-integrons (class 4) are made up of several large integrons, themselves localised on the chromosome and carrying cassettes encoding proteins with a variety

of functions [18]. Class 1 integrons are very widespread. They have been described mainly in enterobacteria and other Gram-negative bacteria in humans, animals, and environment strains [19]. These integrons have an ORF1 open reading frame, the presence of which increases the efficiency of the first cassette [20]. Class 2 integrons have a similar structure to those of class 1 and are associated with the Tn7 transposon. This transposon contains three resistance cassettes: *dfrA1* conferring resistance to trimethoprim, *sat* to streptothricin and *aadA1* to streptomycin and spectinomycin. Class 2 integrons have also been found in Gram-negative bacillus strains of human, animal, or environmental origin [19]. The class 3 integron, which has 61% homology with *intI*1, also consists in its 5 regions of an *intI* gene associated with a promoter and recombined at an *attI* site. It also has an *attI3* recombination site and two promoter regions [19].

Thus, in order to assess the prevalence and distribution of antibiotic resistance determinants in non-cultivable bacterial communities of environmental origin, the search for class 1, 2 and 3 integrons by PCR associated with betalactam family ARGs was performed on total DNA extracts from wastewater samples.

2. Materials and Methods

2.1. Sampling Stations and Sampling Strategies

2.1.1. Description of the Sampling Stations

During the survey campaigns, 14 sampling points were selected in artificial open channels receiving urban wastewater. These 14 points are distributed in 4 sectors described in **Figure 1**.

All these urban's towns have drainage channels whose natural outlet is surface water. The sampling sites were chosen in the main canals according to the anthropogenic activities producing industrial, hospital, traditional slaughterhouse, commercial and household wastewater. For the city of Abidjan, the northern part (Abidjan North), the Zoo-HMA, Williamsville, Fraternité and Corniche sites were identified. For the southern part (Abidjan South), the sites of Saco, Marcory, Sicogi 1 and Sicogi 2 were chosen. For the city of Bouaké, the sites identified were the Gonfreville station, the Diambrou station and the Bouaké University Hospital (CHU) station. In Yamoussoukro, the sites identified were Yakro pharmacy, Yakro mosque and Yakro sheep.

A total of 76 samples were collected between 20 January 2019 and 7 October 2020 from 8 sampling campaigns conducted on different dates in Abidjan North (n = 32) and 5 in Abidjan South (n = 20), during which one sample was collected for each location. For the two cities of Bouaké and Yamoussoukro, two campaigns were carried out. At the end of these campaigns, 4 samples were collected per station.

2.1.2. Sampling

One (1) litre of water was collected in a sterile and carefully labelled polypropylene bottle. These ones were rinsed before the sampling phase with the water to

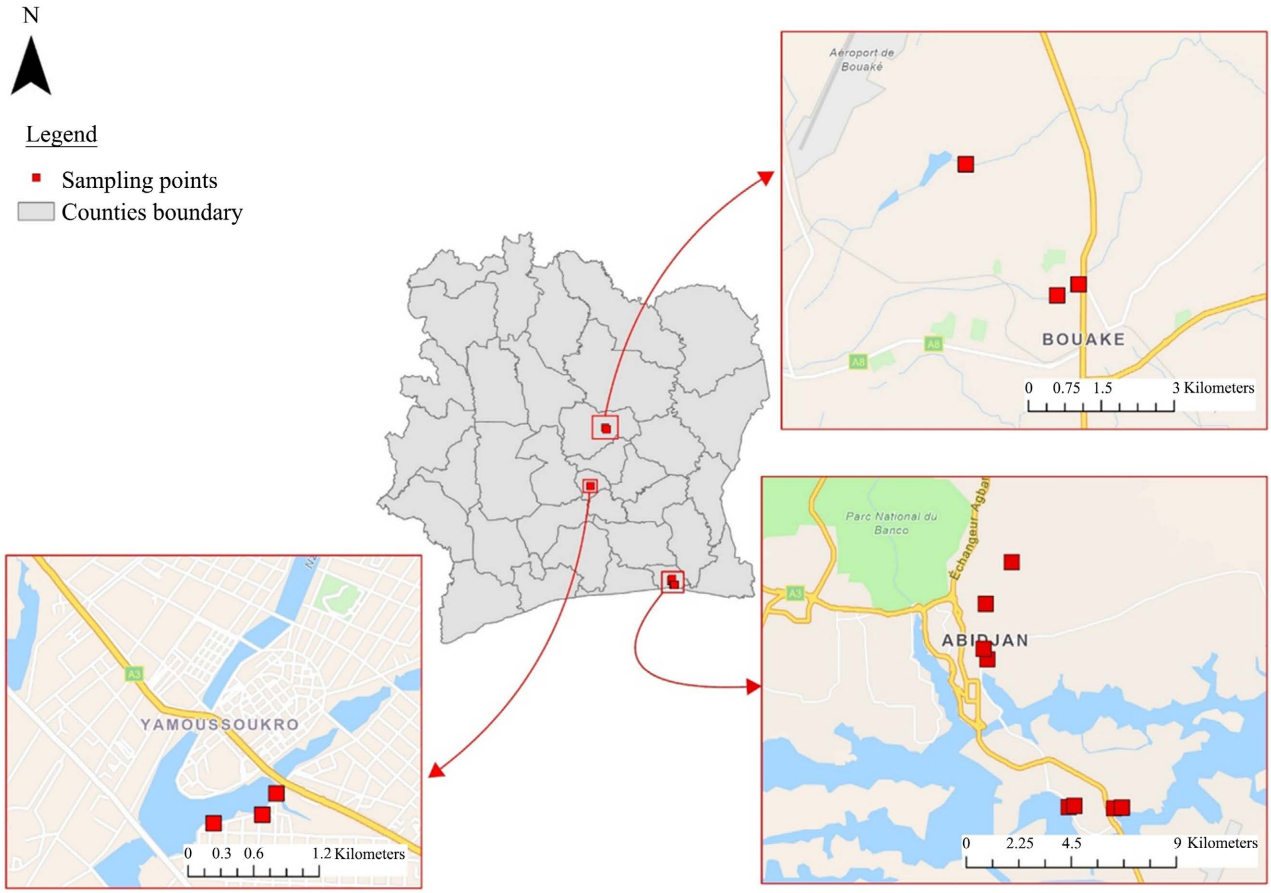


Figure 1. The different sampling sites in the major cities of Abidjan, Bouaké and Yamoussoukro.

be sampled. Bottles containing water sampled have been kept in a cooler maintained at a low temperature (4˚C), and then immediately transported to the laboratory to be processed within 6 hours of collection. Sampling is carried out under strict aseptic conditions to avoid any contamination during handling.

2.2. Search for Genetic Markers and Antibiotic Resistance Genes

2.2.1. Extraction of Total Environmental DNA

A volume of 30 - 50 mL of water was filtered (in triplicate) to concentrate the bacterial biomass on sterile cellulose acetate membranes with a porosity of 0.22 µm diameter (Prat Dumas France) for the collected samples. All recovered membranes were stored at −20˚C for further extraction. A total of 39 of the 76 filters from the canals were selected for the study, giving a positivity rate of 51.31%. Volume quantities were filtered so that there was an average of two filters for each sampling point.

Total DNA extraction was performed using the Masterpure Gram-positive Bacterial DNA Extraction Kit (Epicentre®). The protocol was modified to obtain sufficient total DNA extracts for amplification of the target genes. The filters were placed in a tube containing 3 mL of water for injection (WFI). The mixture was vortexed for 5 min at maximum speed in order to detach the elements on

the surface and then centrifuged at 12,000 g to precipitate the bacteria at the bottom of the tube (this step was repeated 3 times by taking 1 mL of mixture). Once the pellet was obtained, the DNA was extracted by enzymatic lysis. The final concentration and purity were determined using a NanoDrop spectrophotometer (NanoDrop one thermofisherâ). The DNA extracts are stored at −20˚C for resistance gene testing.

2.2.2. Polymerase Chain Reaction: PCR

The study focuses on the search for class 1, 2, 3 integrons (encoded by the *intI*1 (Integron 1), *intI*2 (Integron 2) *and intI*3 (Integron 3) genes, each specific to a class of integrons) frequently associated with genes conferring antibiotic resistance (ARGs) such as the *blaTEM*, *blaSHV and blaCTX-M genes* encoding *β*-lactamases and the *blaKPC*, *blaNDM*, *blaOXA48*, *blaIMP and blaVIM* genes encoding carbapenemases (Table 1). These genes are sought in all total DNA extracts from non-cultivable bacterial communities. Resistance integrons used as genetic markers for the spread of antibiotic resistance will be screened in total DNA extracts. Amplification is performed in a total reaction volume of 25 µL with 0.5 µL of DNA (adjusted to 1 µL for filters), 0.2 mM dNTP, 1× buffer, 2.5 mM Magnesium Chloride ($MgCl_2$), 0.2 µM of each primer specific to the desired gene (Table 1) and 0.05 U of Taq Polymerase (Gotaq G2 Flexi DNA Polymerase, Promega). The amplicons were migrated onto a 1.5% agarose gel for 35 min at 100 volts and then visualised under UV. For integrons, only simplex PCR was performed, which allowed them to be differentiated on different gels.

2.3. Statistical Analysis

Statistical analyses were performed using Excel 2016 and Python software and libraries. QGIS software was used to elaborate the maps and integrate the results of the specific PCRs.

3. Results

All total DNA extracts from the 39 wastewater sample filters were screened by conventional PCR for the presence of genetic markers for class 1, 2, 3 integrase genes and beta-lactam resistance genes.

PCR identified class 1 integrons (Figure 2(a)), class 2 integrons (Figure 2(b)) and class 3 integrons (Figure 2(c)) in the total DNA extracts.

3.1. Determination of Genetic Markers for Resistance and AMR

The results for the search for genetic markers for class 1, 2, 3 integrons are shown in Table 2 and for genes conferring betalactam resistance are shown in Table 3.

The data presented correspond to the number of positives among the filters of processed samples (n = 39). Class 1 integron is most evident in the samples (present in 94.8% of samples) in Abidjan (93.3%), Bouaké (100%) and Yamoussoukro

Table 1. Oligonucleotides and PCR conditions of the study (F: sense primer; R: antisense primer).

PCR	Genes Cibles	Sequences Amorces (5'-3')	Size of the Amplicon (pb)	Sources
Search for integrons	*intI*1	F: 5'-GCCTTGATGTTACCCGAGAG-3' R: 5'-GATCGGTCGAATGCGTGT-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 60˚C/30s, 72˚C/30s) × 35; 72˚C/7min	196	[21]
	*intI*2	F: 5'-TGCTTTTCCCACCCTTACC-3' R: 5'-GACGGCTACCCTCTGTTATCTC-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 62˚C/30s, 72˚C/30s) × 35; 72˚C/7min	195	[21]
	*intI*3	F: 5'-GCCACCACTTGTTTGAGGA-3' R: 5'-GGATGTCTGTGCCTGCTTG-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 62˚C/30s, 72˚C/30s) × 35; 72˚C/7min	138	[21]
Searching for genes encoding ESBL	*blaTEM*	F: 5'-GCKGCCAACTTACTTCTGACAACG-3' R: 5'-CTTTATCCGCCTCCATCCAGTCTA-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 60˚C/30s, 72˚C/30s) × 35; 72˚C/7min	247	[22]
	blaSHV	F: 5'-CGCTTTCCCATGATGAGCACCTTT-3' R: 5'-TCCTGCTGGCGATAGTGGATCTTT-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 60˚C/30s, 72˚C/30s) × 35; 72˚C/7min	110	[22]
	blaCTX-M	F: 5'-CTATGGCACCACCAACGATA-3' R: 5'-ACGGCTTTCTGCCTTAGGTT-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 62˚C/30s, 72˚C/30s) × 35; 72˚C/7min	103	[23]
Search for carbapenemase coding genes	*blaNDM*	F: 5'-GATTGCGACTTATGCCAATG-3' R: 5'-TCGATCCCAACGGTGATATT-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 60˚C/30s, 72˚C/30s) × 35; 72˚C/7min	189	[24]
	blaKPC	F: 5'-CAGCTCATTCAAGGGCTTTC-3' R: 5'-GGCGGCGTTATCACTGTATT-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 62˚C/30s, 72˚C/30s) × 35; 72˚C/7min	196	[24]
	blaIMP	F: 5'-GGAATAGAGTGGCTTAAYTCTC-3' R: 5'-GGTTTAAYAAAACAACCACC-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 56˚C/30s, 72˚C/30s) × 35; 72˚C/7min	200	[24]
	blaVIM	F: 5'-GATGGTGTTTGGTCGCATA-3' R: 5'-CGAATGCGCAGCACCAG-3' PCR Cycle: 94˚C/2min; (94˚C/1min, 58˚C/30s, 72˚C/1min) × 35; 72˚C/7min	390	[24]
	*blaOXA*48	F: 5'-AGGCACGTATGAGCAAGATG-3' R: 5'-TGGCTTGTTTGACAATACGC-3' PCR Cycle: 95˚C/3min; (95˚C/30s, 60˚C/30s, 72˚C/30s) × 35; 72˚C/7min	189	[24]

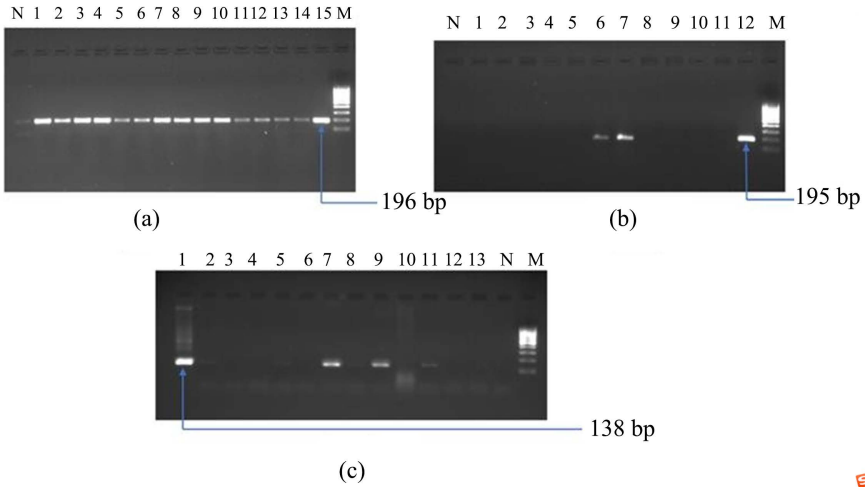


Figure 2. Electrophoretic profile of class 1, class 2 and class 3 integron gene carriers isolated from total DNA extracts from the filters. (a): PCR products of the class 1 integrase gene (*intI1*), M-track; molecular weight marker (*Euromedex DNA Ladder 100* bp); Tracks 1 - 14: no model control (negative); tracks 15: positive control (Intl1). (b): PCR products of the class 2 integrase gene (*intI2*). Track M: Molecular weight marker (*Euromedex DNA Ladder of 100 bp*); Tracks 1 - 11: no pattern control (negative); tracks 12 positive control (Intl2). (c): PCR products of the class 3 integrase gene *intI3*. Track M: molecular marker; Tracks 2 to 13: no model check (negative); track 12 positive control (Intl2).

Table 2. Distribution of class 1, 2, 3 integrons in wastewater samples from different sites.

Sites	Genetic Markers		
	Class 1 Integron	Class 2 Integron	Class 3 Integron
North Abidjan (N = 8)	7	0	0
South Abidjan (N = 7)	7	2	0
Bouaké (N = 12)	12	4	10
Yamoussoukro (N = 12)	11	10	10
Grand total (N = 39)	**37**	**16**	**20**
Detection rate (%)	**94.87**	**41.02**	**51.28**

Table 3. Distribution of resistance genes encoding *β*-lactamases (*blaTEM*, *blaSHV*, *blaCTX-M*) *and* carbapenemases (*blaKPC*, *blaNDM*, *blaIMP*, *blaVIM*, *blaOXA48*) in wastewater samples from different sites.

Sites	Genes Encoding *β*-Lactamases			Genes Encoding Carbapenemases				
	TEM	SHV	CTX-M	KPC	NDM	OXA 48	IMP	VIM
North Abidjan	8	4	0	3	3	4	0	0
South Abidjan	7	3	3	3	2	3	0	0
Bouaké	12	9	3	1	3	2	0	0
Yamoussoukro	11	10	5	0	4	6	0	0
Grand total	**38**	**26**	**11**	**7**	**12**	**15**	**0**	**0**
Detection rate (%)	**97.43**	**66.66**	**28.2**	**17.94**	**30.76**	**38.46**	**0**	**0**

TEM: TEMoneira; **SHV**: SulfHydryl Variable; **CTX-M**: CefoTaXimase-Munich; **KPC**: *Klebsiella Pneumoniae* Carbapenemase; **NDM**: New-Dehli Metallo-*β*-lactamase; **OXA 48**: OXAcillinase; **IMP**: Imipenemase; **VIM**: Verona Integron encoded Metallo-*β*-lactamase.

(91.6%). Class 2 integrons were found in only 41% of the samples, in only 7 sites out of 14, and class 3 integrons were identified in Yamoussoukro (83.33%) but never in Abidjan. Concerning the genes coding for *β*-lactamases, blaTEM was detected in almost all samples (97.4%). A co-presence of the three genes blaTEM, blaSHV and blaCTX-M is also remarkable at the Yakro pharmacie, Yakro mosque and Yakro mouton sites in Yamoussoukro. They are also found in at least one of the samples from Diambrou (Bouaké) and Abidjan Sud.

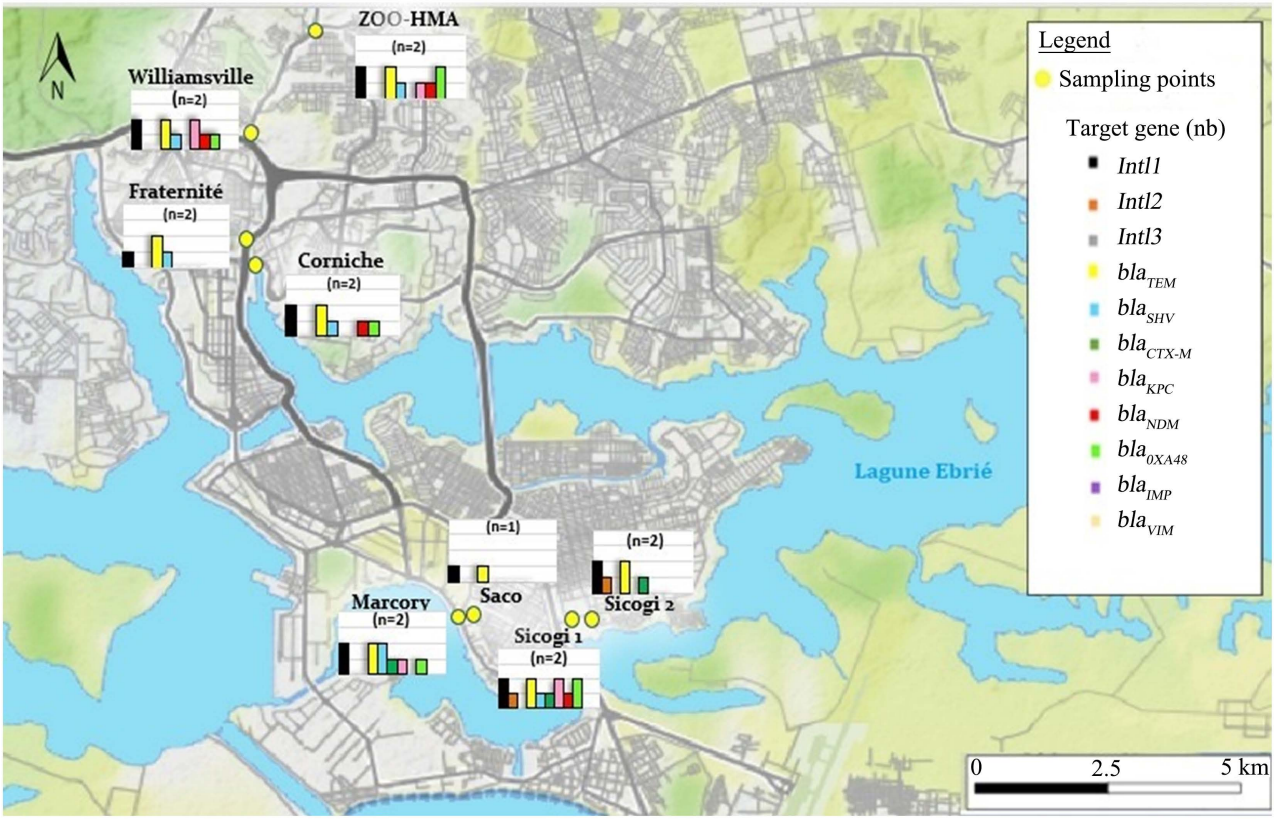
3.2. Spatial Distribution of Genetic Markers and AMR

Mapping of class 1, 2, 3 integrons and resistance genes encoding *β*-lactamases (*blaTEM*, *blaSHV*, *blaCTX-M*) and carbapenemases (*blaKPC*, *blaNDM*, *blaIMP*, *blaVIM*, *blaOXA48*)

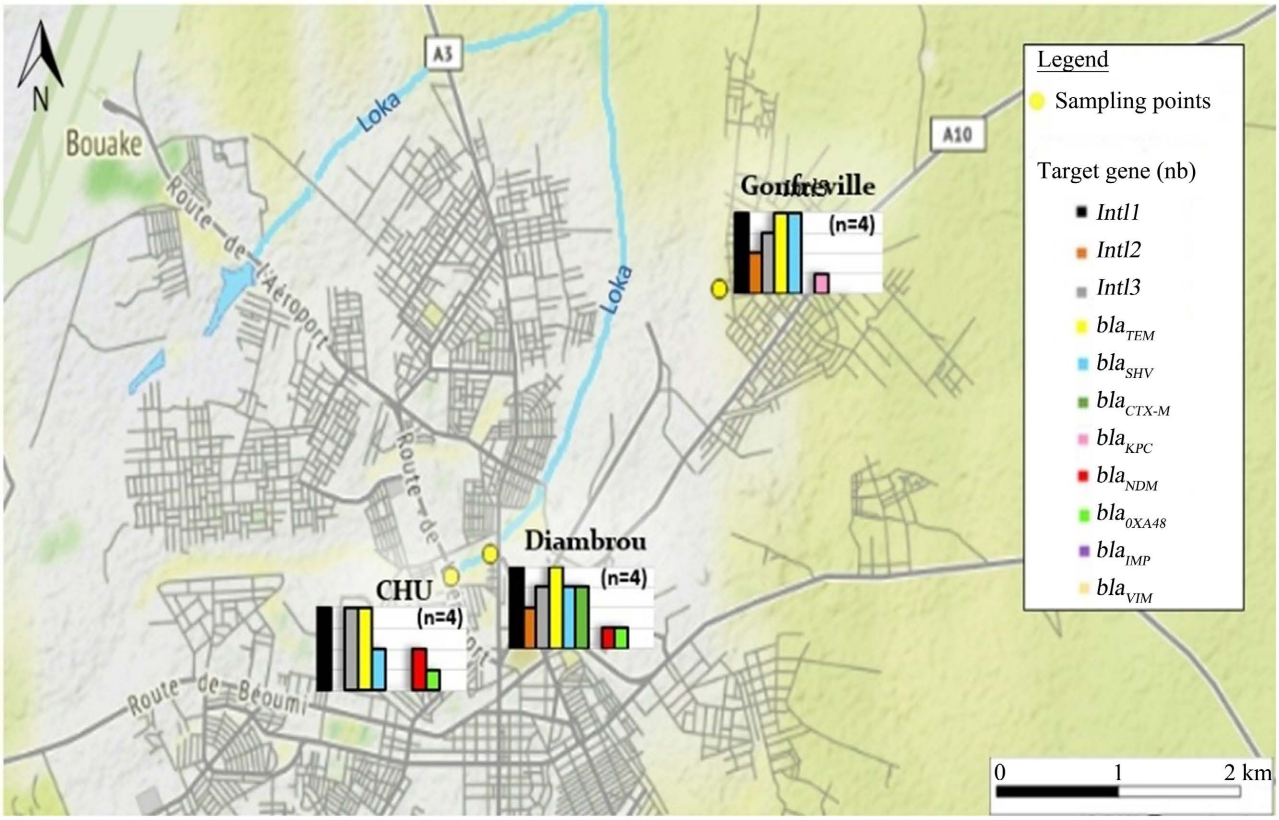
Spatial dynamics were studied at 14 sites in 3 cities (Abidjan, Bouaké and Yamoussoukro). The integrons were distributed according to the sites on a map (Figure 3). The mapping of genetic markers associated with the ARGs found in the samples for each site is shown in Figure 3.

3.3. Identifying Correlations between Genetic Markers of Resistance and AMR

The heat map shows the different relationships between the presence of genetic markers and *β*-lactam resistance genes. For the class 1 integron resistance marker we find a strong correlation with the blaTEM gene (0.97) followed by blaSHV (0.77). For the class 2 integron, there is a strong correlation with the blaSHV, blaTEM and blaCTX-M genes. The class 3 integron has a strong correlation with the blaTEM and blaSHV genes. For the genes coding for carbapenemases, there is a weak correlation between the resistance markers and the KPC, NDM and OXA-48 genes. The IMP and VIM genes were not detected in these samples (Figure 4(a)). Indeed, the presence of resistance markers is strongly associated with the circulation of genes coding for betalactamases. Class 1, 2 and 3 integrons are strongly associated with blaSHV, blaTEM and blaCTX-M. However, the presence of resistance markers does not influence the presence of carbapenemase genes. The hierarchical clustering dendrogram allowed us to see where antibiotic resistance is most prevalent among the 14 sites. Indeed, the dendrogram made it possible to group all the genetic determinants into three groups according to the presence or absence of all the resistance markers with the set of genes coding for betalactamases and carbapenemases. This suggests that the city of Yamoussoukro, particularly the Yakro Mouton site, is the one most affected by a strong dissemination of antibiotic resistance. After the city of Yamoussoukro, the most impacted cities are Bouaké and Abidjan (Figure 4(b)). These data confirm the spatial distribution of sites with high diffusion of resistance determinants. We note that the site of Yakro mouton, followed by the sites of Bouaké and Yamoussoukro, are strongly impacted by the diffusion of resistance markers and AMR (Figure 4(b)).


N
ZOO-HMA
(n=2)
Williamsville
(n=2)
Fraternité
(n=2)
Corniche
(n=2)
Lagune Ebrié
(n=1)
Saco
(n=2)
Sicogi 2
Marcory
(n=2)
Sicogi 1
(n=2)
Legend
Sampling points
Target gene (nb)
Intl1
Intl2
Intl3
bla TEM
bla SHV
bla CTX-M
bla KPC
bla NDM
bla 0XA48
bla IMP
bla VIM
0
2.5
5 km

(a)


N
A3
Loka
Bouake
Route de l'Aéroport
A10
Gonfreville
(n=4)
Loka
Route de
Diambrou
(n=4)
CHU
(n=4)
Route de Béoumi
Legend
Sampling points
Target gene (nb)
Intl1
Intl2
Intl3
bla TEM
bla SHV
bla CTX-M
bla KPC
bla NDM
bla 0XA48
bla IMP
bla VIM
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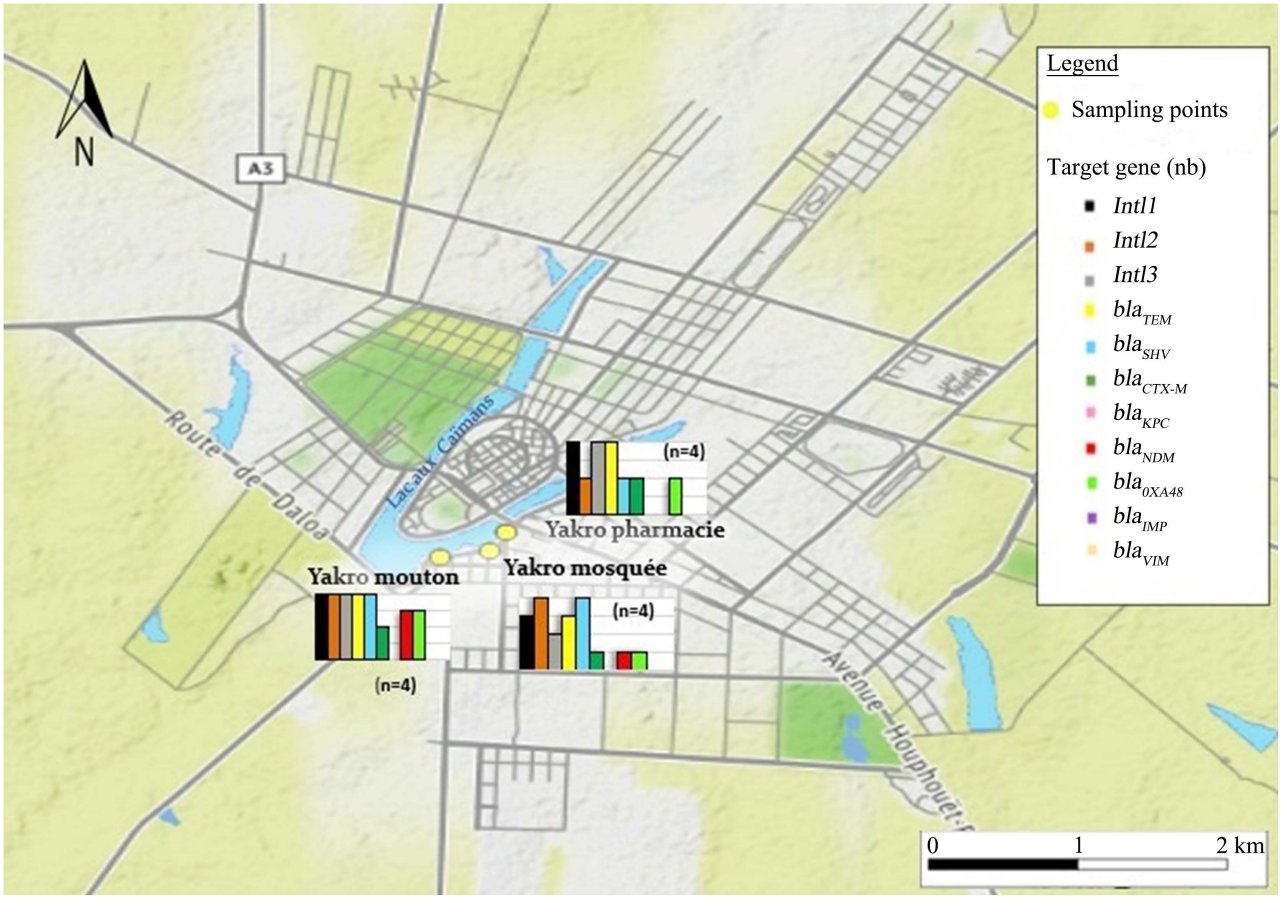
1
2 km

(b)



(c)

Figure 3. Geographical representation of class 1, 2, 3 integrons, resistance genes of β-lactamases (*blaTEM*, *blaSHV*, *blaCTX-M*) and carbapenemases (*blaKPC*, *blaNDM*, *blaIMP*, *blaVIM*, *blaOXA48*). (a) City of Abidjan, (b) City of Bouake, (c) City of Yamoussoukro.

4. Discussion

The cities of Côte d'Ivoire are increasingly confronted with the deterioration of their environment, notably due to untreated wastewater discharged into the aquatic environment and thus contributing to the dissemination of antibiotic resistance as indicated by an ANSES study in France [25]. According to their work on the sources of environmental contamination by antibiotics, resistance genes may persist for longer, either outside the cells or hosted by other bacteria not taken into account in studies [25] [26]. To this end, the present work focused on the search for genetic markers and AMR from environmental DNA extracts. The prevalence of *Intl1*, *blaTEM*, *blaSHV*, *NDM and OXA*-48 genes at all sites (drainage channels or liquid effluents) was demonstrated. In order to assess the potential for dissemination of antibiotic resistance from urban wastewater into the environment, resistance integrons were monitored as genetic markers and for the acquisition of antibiotic resistance expression as showed by [27] and [28]. Resistance integrons were found in all total DNA extracts from the sample filters (non-cultivable bacterial community). This generally confirms that resistance

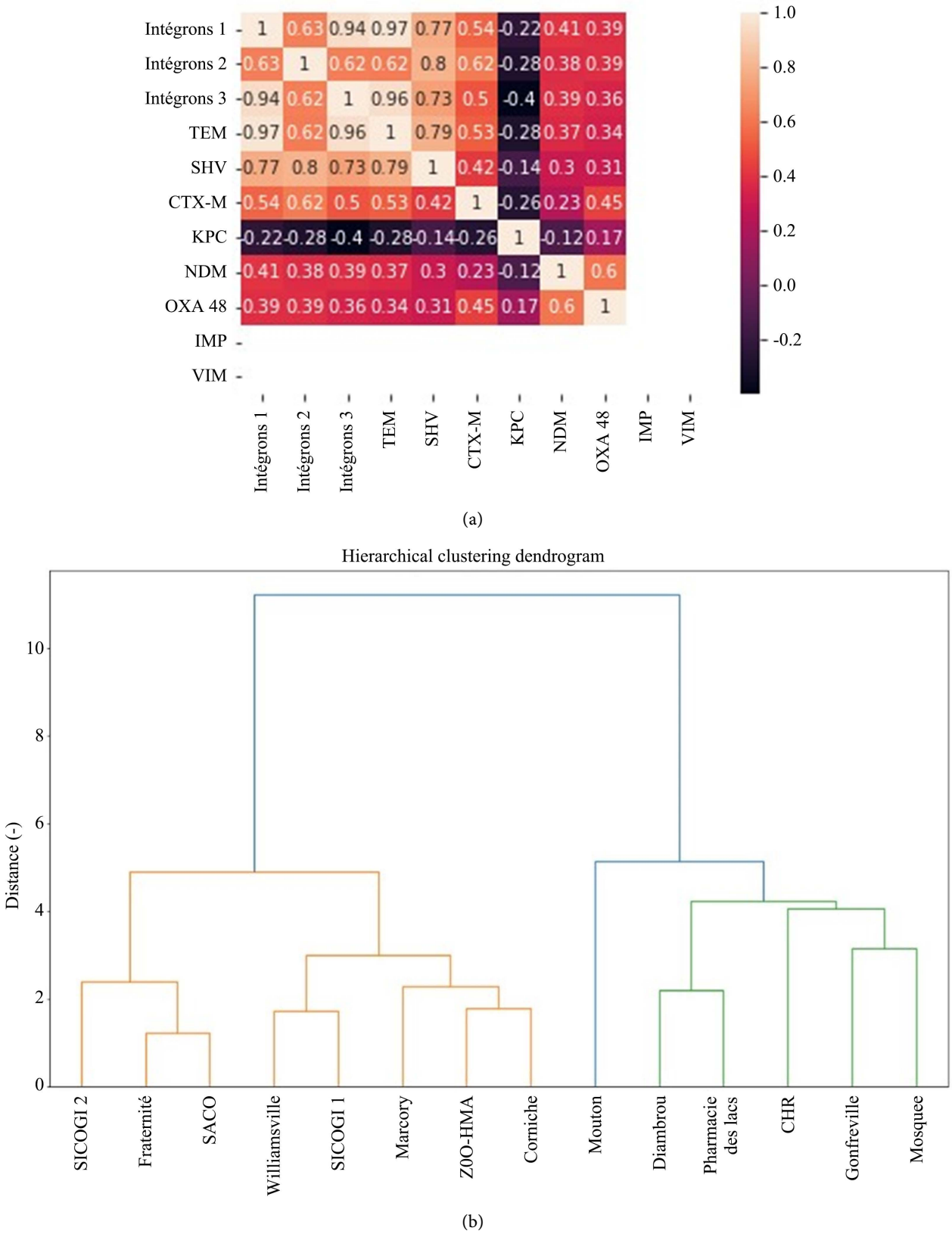


Figure 4. Spatial distribution of genetic determinants by site. (a) heat map representation and correlations between genetic resistance markers and ARMs; (b) Hierarchical clustering dendrogram according to the presence of genetic resistance markers and ARMs in relation to the sampled sites.

integrons are often proposed as an anthropogenic indicator due to the epidemiological link between their presence in bacterial communities and waterborne multidrug resistance [29]. Indeed, resistance integrons are found in a large number of bacterial species. They affect both Gram-negative and Gram-positive bacteria, as well as clinical and environmental bacteria [30]. Resistance integrons are often associated with mobile genetic elements such as plasmids or transposons. Therefore, they are widely implicated in the spread of Antibiotics resistance in bacteria [26] [31]. In total DNA extracts from non-cultivable bacterial communities, class I integrons and class III integrons have a very high prevalence respectively, followed by class II integrons with a medium rate. The presence of antibiotic resistance genes in the wastewater of certain towns in Côte d'Ivoire and the higher prevalence of Class 1 integrons compared to others, particularly Class 2 integrons, has also been demonstrated in other African countries, particularly Nigeria, by the studies of Adelowo *et al.* [32]. These results show that African urban ecosystems impacted by anthropogenic activities are reservoirs of bacteria harbouring transferable ARG. [33]. Indeed, class I integrons are the most prevalent integrons in the clinic as described by Malek *et al.* [34] and are therefore the most studied. They have been described in many Gram-negative bacteria (BGN) and more rarely in Gram-positive bacteria (BGP). Most cassettes have already been described within class 1 integrons [35]. Thus, there is a multitude of different cassette arrays and as many as 3000 class 1 integron sequences are listed. The *aad* and *dfr* cassettes are particularly represented (in more than 60% of the cassette arrays in *E. coli*, according to INTEGRALL). The presence of class I integrons is strongly correlated with multiresistance phenotypes making them major players in the dissemination of Antibiotic resistance [36]. Class III integrons generally carry a network containing bla (β-lactam resistance) and aacA4 (resistance to tobramycin, netilmicin and amikacin) genes [37]. While the clinical impact of class 3 integrons remains anecdotal, metagenomic approaches have shown significant amounts of class 3 integrons in environmental samples [38]. Class 3 integrons could therefore be important players in the spread of antibiotic resistance in the environment. Much less prevalent than class 1 and 3 integrons, class 2 integrons are often of clinical origin and carry a low cassette diversity which is partly explained by the non-functionality of the IntI2 integrase preventing the capture of new cassettes. As a reminder, the intl2 gene has a premature STOP codon within it [36].

High levels of blaTEM and blaSHV genes and low levels of blaCTX-M genes are linked to the contamination of these waters by anthropogenic activities. Indeed, blaTEM and blaSHV genes are most often identified in strains of animal origin and are responsible for more than 50% of resistance to β-lactamines [39]. In contrast, blaCTX-M genes are predominant in strains of human origin. High levels of blaOxa-48, blaKPC and blaNDM genes conferring resistance to carbapenems are also noted. The persistence of these AMR in urban wastewater would be favoured by their carriage on plasmids that are easily transmitted by horizontal transfer. These results could be explained by a naturally high prevalence of

certain types and subtypes of plasmids within bacterial communities of various origins, which in fact constituted a privileged reservoir of β-lactamase genes and carbapenemases [40]. The abundance of antibiotic resistant strains in the environment suggests that resistance determinants can be maintained in the absence of selection pressure from the presence of antibiotics. However, the reason for this stability remains unclear. One possibility for the maintenance of these resistance determinants is their coupling to other genes conferring different types of selective advantages (resistance to heavy metals, virulence factors, enzymes increasing the colonising capacity of the bacteria, etc.) [41]. The spread of these AMR and resistance markers suggests that urban water environments in these settlements may constitute a reservoir for the spread of antibiotic resistance. The presence of the BlaTEM and BlaCTX genes identified in the wastewater in this study was found by Mbanga *et al.* [42] in their work carried out in South Africa. This clearly shows that untreated wastewater, as in Côte d'Ivoire, but also in emerging countries such as South Africa, can be vehicles for the dissemination of integrons and also antibiotic resistance genes.

Statistical analysis revealed significant positive correlations between the presence of genetic markers, class 1, 3, 2 integrons and genes encoding β-lactamases. These correlations show a link between the presence of resistance integrons and the blaTEM, blaSHV and blaCTX-M genes. In contrast, there was a low significant correlation for the association of genes encoding carbapenemases. This reflects the nature of the various effluents discharged into the water environment, which exerts strong selection pressure and favours genetic exchanges between the bacterial communities present in these waters. These correlations made it possible to cluster according to the presence of genetic markers and AMR across the 14 sampling sites in urban wastewater. Thus, a hierarchical distribution of risk sites with a high potential for the spread of AMR could be performed and projected on the factorial plane.

In environmental samples, the class I integron shows considerable genetic sequence diversity, whereas the same gene from anthropogenic sources shows uniform and conserved sequences [43]. The amplification of this gene allows us to state that all study sites are under anthropogenic pressure.

Indeed, at the scale of the city of Abidjan, the Fraternité and Saco sites are characterised by the presence of a lower number of ARGs in the samples compared to the other sampling sites. The Fraternité site is certainly distinguished by its location and the nature of the water it drains. It is located on a tributary of the main canal crossing the Zoo-HMA, Williamsville and Corniche sites and drains water from a neighbourhood in the commune of Adjamé. As for the Saco site, it receives discharges of industrial origin.

In addition, a significant presence of genes encoding ESBL and carbapenemases was found in the different sites. Firstly, along the same channel, as for example in Abidjan Nord, they were amplified in the samples in Zoo-HMA, Williamsville and Corniche. Secondly, in the Yamoussoukro and Bouaké sites,

all the genes were found to be abundant, particularly in the separate canals carrying the domestic water of the city of Yamoussoukro (Yakro pharmacy, mosque and sheep). In the Yamoussoukro and Bouaké sites, all the genes were found to be abundant, particularly in the separate canals carrying the domestic water of the city of Yamoussoukro (Yakro pharmacy, mosque and sheep). The diverse nature of the effluents that feed these canals does not allow us to rule on an exact source of contamination. However, we know that the Zoo-HMA site is adjacent to a hospital which discharges its effluents without prior treatment. The Sicogi 1 site, where the presence of the 3 genes coding for β-lactamases and carbapenemases was noted, is supplied by water of industrial origin. The discharge of water containing trace metals (TMEs), such as Cd, Cu, Hg or Zn by a nearby factory could explain these results. Indeed, TMEs can selectively induce the co-selection of antibiotic resistance if they spread and accumulate in the environment at critical concentrations [44].

5. Conclusion

Our wastewater study provided data on resistance genes circulating in Côte d'Ivoire. The circulation of such genes leads to the fear of a transfer of resistance to waterborne strains allowing their maintenance in the environment and their dissemination, or a return of antibiotic resistance in the clinical environment by transferring genes to strains that are potentially pathogenic for humans. These results show that a major effort must be made in terms of water treatment. It is essential that sanitation systems are put in place to treat the effluent of the cities studied in order to protect human, animal and environmental health. The appropriate use of antibiotics can help stop the emergence of resistance genes and multi-resistant bacteria in the environment, thus ensuring that the latest molecules in our therapeutic arsenal are available for future generations. The local population and prescribers should therefore be made aware of the sensible use of antibiotics. In order to monitor the ecological cycle of antibiotic resistance, it would be interesting to quantify the presence of the genes studied in order to assess the degree of the anthropisation of the environments at risk identified.

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Kalpy J. Coulibaly: Conceptualization, Methodology, Data curation. **Aboubakar S. Diaby**: Visualization, Investigation, Writing-Original draft preparation. **Sabine N. Vakou**: Data curation, Investigation. **Valérie C. Gbonon**: Supervision. **Jean S. Claon**: Investigation, Data curation. **Eric K. Yao**: Investigation. **Gnali G. Fabrice:**, Investigation, Sampling. **Yéo Yéfougnini**: Read and corrected the first draft. **Issa Bagré**: Supervision, Reviewing. **Joseph A. Djaman**: Supervision, Reviewing. **Mireille Bretin Dosso**: Reviewing and Editing.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data are available at Institute Pasteur de Côte d'Ivoire and with authors. Authors are ready to share on demand at any moment.

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