



Prevalence of Antibiotics Resistant *Salmonella* in the Abidjan North Wastewater in 2012 and Potential Health Risks to the Population

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Authors' contributions

This work was carried out in collaboration between all authors. Author CKJ designed the study, wrote the protocol and first draft of the manuscript. Authors KKS and DS managed the analyses of the study. Author BA managed the sampling. Author KDA corrected the protocol. Author DM supervised the analysis. All authors read and approved the final manuscript.

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ABSTRACT

Salmonella cause salmonellosis, a disease which most common symptoms include diarrhea, abdominal cramps, and fever.

Aims: The purpose of this study was to highlight the health risks linked to *Salmonella* for the population through the study of wastewater in the Indénié wastewater discharge adjacent to major effluents in the Gourou Basin.

Materials and Methods: From June to August 2012, wastewater samples were collected from two selected reservoir adjacent to the Gourou Basin collectors at the crossroads of Indénié in Abidjan. About 500 mL of collected wastewater was used to search for the bacteria of the genus *Salmonella* according to the standard NF EN ISO 6579. The identification was made using the API 20E gallery

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and confirmed by MADITOFF-MS. Bacteria strains serotyping was performed and the Kirby Baeur disc diffusion method was used for the antibiotic and susceptibility study.

Results and Discussion: A total of 112 samples were collected and were used to isolate 18 strains of *Salmonella*. Serotyping showed 8 different serotypes with a predominance of *Salmonella* Typhimurium and *Salmonella* Poeseldorf. The prevalence of *Salmonella* resistant to antibiotics in this study was 4.46%. About 27.80% of antibiotic resistant *Salmonella*, were resistant to at least one antibiotic. *Salmonella* strains were all sensitive (100%) to 3rd generation cephalosporins (C3G), 16.67% of resistant strains showed cross-resistance to quinolones. In this study, it was demonstrated the biological risks involved by the presence of *Salmonella* in the wastewater. The prevalence rate and the serotype highlighted were different from that of similar study conducted in Africa.

Conclusion: The wastewater contamination by *Salmonella* may constitute a risk of diffusion of antibiotic resistant *Salmonella* in the population.

Keywords: *Salmonella*; wastewater; antibiotic resistance; health risk.

1. INTRODUCTION

Discharge of untreated wastewater into the environment can be a source of chemicals and microbials pollutions. It could also induce infections because of the presence of pathogenic microorganisms of diverse origins [1,2,3,4].

According to the World Health Organization (WHO) in 2004, each year about 1.8 million people of which 90% are children under the age of five (5) die from diarrheal diseases. In general the vast majority of diarrheal disease is attributed to the poor quality of drinking water and the absence of sewage treatment [5,6].

So Wastewater management has become an imperative for modern societies, but African countries are experiencing serious difficulties which are water supply and wastewater disposal and treatment. It is noted that 78% of African cities have no formal regulations for the treatment of wastewater and disposal management and where they exist, they are either outdated or inadequate in most cases [7].

In Cote d'Ivoire, the government as part of her infrastructure development policy constructed two wastewater receiving canals to regulate their flow in different neighborhoods in the north of the city of Abidjan [8]. These receiving canals are products of the integrated management project of the basin of Gourou [9]. Unfortunately, these canals are poorly maintained and fail to play their primary role, thus causing a threat to public health.

In many countries, the monitoring of wastewater quality when performed is limited to the estimation of the level of pollution indicator

microorganisms. However, many studies have shown that the presence of indicator bacteria is not always correlated to the presence of pathogenic organisms [10]. Eventhough majority of bacteria present in the wastewater are not pathogenic, some pathogens such as *Salmonella* have been involved in waterborne diseases mainly in developing countries. The increase of pollution in natural water has intensified the detection frequency and persistence of pathogenic microorganisms mainly *Salmonella* in areas affected by sewage discharge [11]. So European Union (EU) reported that *Salmonella* must be checked in addition to the indicators. That is not the case in other places. Authors have claimed that intermittent presence of *Salmonella* has been seen as a result of concrete case contamination [11].

The presence of these bacteria in wastewater is sometimes the consequences of human activities such as hospital, industrial and livestock effluents [12]. It could also be due to the environmental action (wild bird, others animals and others sources) [13]. Most *Salmonella* are pathogens to humans and are often used as biological risk markers [14]. *Salmonella* causes a serious health problem in developing countries through a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia but also through the increasing of their resistance to antibiotics [11]. *Salmonella* can received genetic elements coding for antibiotics resistance in wastewater [4]. The increased antibiotics resistance of *Salmonella* is a serious threat to public health.

Other studies have revealed as part of public health, that the analysis of wastewater can be seen as a simple method of epidemiological surveillance of enteric infections [2,15,16,17].

Indeed, when this analysis is performed on a regular basis it allows drawing a list of pathogenic enteric bacteria circulating in the population, because the wastewater pathogen load reflects the health status of the population of the region. Surveillance of *Salmonella* in sewage could allow for indirect monitoring of human *Salmonella* [2,18,19].

It was decided to perform this bacteriological study with the main objective to highlight the health risks linked to *Salmonella* in the populations through the analysis of the wastewater in the Indenié receiving canals adjoining to the large collectors of the Gourou Basin.

2. MATERIALS AND METHODS

2.1 Study and Sampling Site

Wastewater samples were collected from the reservoirs adjacent to the collectors of the Gourou Basin. In this study, the analysis were performed at the Pasteur Institute of Ivory Coast in the Chemical and Environmental Microbiology department.

Samples were taken from reservoirs in the Gourou Basin. Gourou Basin covers an area of approximately 28.6 km². It stretches from north to south about 9 km. With an average width of 3 km, is bounded in the east by the extension of the boulevard Latrille towards II Plateaux District and in the west by the rail line (Adjamé-Anyama) [20]. In this present study, we focused on two wastewater reservoirs, one close to the Abidjan Technical High School (Reservoir A) and the other close to the Fraternité Matin Newspaper head office (Reservoir B): (Fig. 1).

Reservoir A (05° 23' 13,6"N et 04° 00' 22,0"W), receives urban wastewater originating from Abovo South, Williamsville, military camp Agban, Cocody II Plateau and Cocody-Technical High School (Fig. 2).

Reservoir B (05 ° 22 '42,7"N and 04 ° 00' 20,6"W) receives urban wastewater from the Principal Mosque Adjamé-Gare, Adjamé-Bracodi, Adjamé- 220 apartments, Plateau, Indenié and Fraternité-Matin area. The drainages that feed these reservoirs and receive garbage that are thrown directly there and wastewater from unidentified drainage channels (Fig. 3).

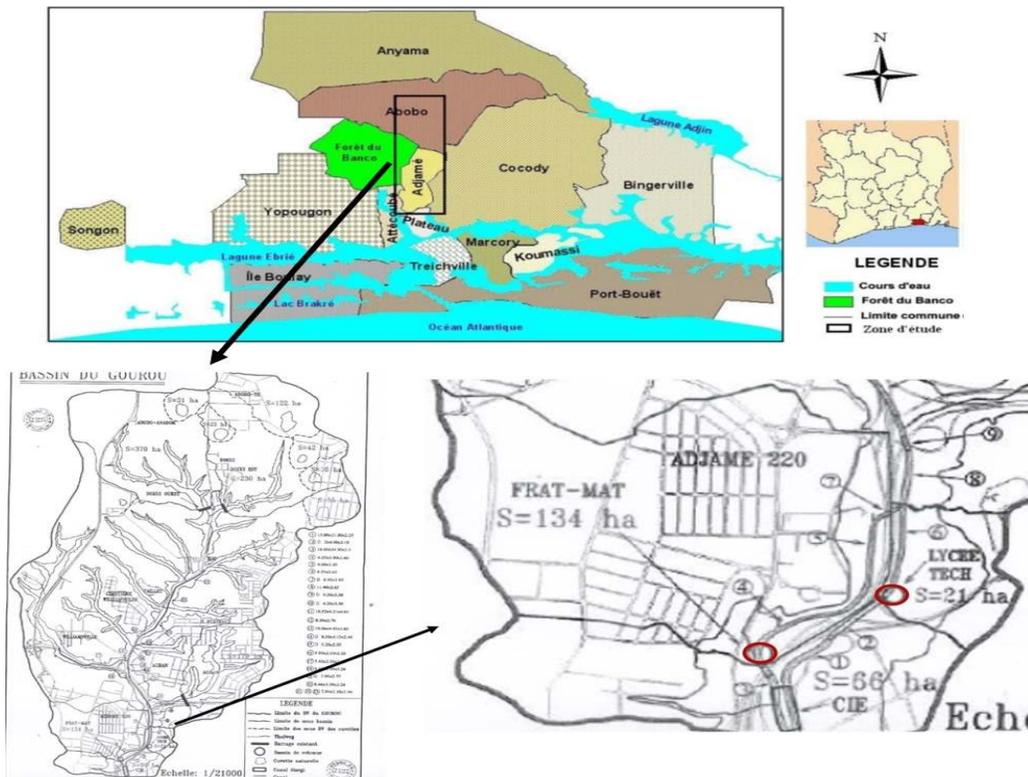


Fig. 1. Map of the Gourou Bassin and the reservoirs studied. (Photo: Kalpy J. Coulibaly)

2.2 Methods

2.2.1 Sampling methods

Sampling were made in the two selected reservoirs mentioned above. However, points were chosen due to their accessibility.



Fig. 2. Overview of the reservoir A located at the Lycee Technique



Fig. 3. Overview of the reservoir B located at the Fraternité Matin

Sample collection was done twice per week over a period of two month (June 2, August 2, 2012). Wastewater was collected in 500 ml pre-sterilized glass bottles. The samples were transported to the laboratory in coolers having ice packs at a temperature close to + 4°C.

2.2.2 Laboratory analysis

2.2.2.1 Bacteria Identification

In the laboratory, the samples were left to decant at the laboratory temperature for about 20 minutes. An aliquot of 50 mL for each sample was stored at - 80°C in the biological resources section of the Pasteur Institute of Cote d'Ivoire.

The analyses carried out was to detect *Salmonella* following the established

microbiology standard NF EN ISO 6579. Briefly, the detection of *Salmonella* involves the classic four phases: pre-enrichment, enrichment, isolation and characterization. The sample was homogenized by vigorously shaking of the bottle. One (01) mL of the sample was taken and inoculated into 9 mL of buffered peptone water. The incubation was performed at 37°C for 24 h in an incubator Memmert®. One (01) mL of pre-enrichment solution was inoculated in 9 mL of rappaport-vassiliadis broth (RV) and incubated at 42°C for 24 hours. Then, about 10 µl of the enriched solution was streaked on Hektoen medium and incubated for 24 h at 37°C.

Green translucent colonies or blue-green with or without black center were collected and identified using the API 20E.

The confirmation of the strains was done by Mass Spectrometry Maldi-Toff (Vitek MS-CHCA; Biomérieux®). Quality control was achieved by using standard strains of *E. coli* ATCC8739.

2.2.2.2 Antigen identification (Serotyping)

Salmonella serotypes were determined by the slide agglutination tests with polyvalent immune sera and monovalent (Bio-Rad). The reading of the results was done according to the table of Kauffmann-White [21].

2.2.2.3 Antibiotics Susceptibility test

Antibiotic sensitivity of *Salmonella* strains was investigated following the disc diffusion method according to Kirby-Bauer [22]. The following antibiotics were tested: amoxicillin (AMX), amoxicillin + clavulanic acid (AMC) (30 µg), cefotaxime (CTX) (30 µg), ceftriaxone (CRO) (30 µg), nalidixic acid (NA)(30 µg), ciprofloxacin (CIP) (5 µg), gentamicin (G) (15 µg). The zones of inhibition were interpreted as recommended by the CA-SFM 2015. For quality control, the ATCC standard strains *E. coli* (ATCC 25922) was used.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Prevalence of *Salmonella* isolated wastewater

In this study, from each reservoir, it was collected 56 samples ie a total of 112 samples in 14

campaigns of collect. *Salmonella* was isolated in 16% of samples.

3.1.2 Distribution of *Salmonella* serotype recovered from reservoir A and B

In this study, the most frequent serotypes isolated were *Salmonella* sp (22,22%), *Salmonella* Typhimurium, *Salmonella* Poeseldorf and *Salmonella* Kiel with 16,67% for each serotype. All *Salmonella* Typhimurium and *Salmonella* Poeseldorf were found in the reservoir A while the *Salmonella* Kiel was found both in the two reservoir. Only one (5,56%) strain was isolated for the other serotypes. It was the case for *Salmonella* Nitra, *Salmonella* Eppendorf, *Salmonella* Elisabethville, *Salmonella* Illb and *Salmonella* Stockholm.

Salmonella Stockholm and *Salmonella* Eppendorf were isolated in the reservoir B and the others in Reservoir A. Overall, eight serotypes have been identified. Six (06) different serotypes were isolated in the Reservoir A against three (03) in the Reservoir B. Only *Salmonella* Kiel was common to the 2 reservoir (Fig. 4).

3.1.3 Antibiotics resistance profiles of *Salmonella* strains

The prevalence of *Salmonella* resistant to antibiotics in this study was 4.46%. Out of the 18 *Salmonella* isolates of our study, 13 strains (72.20%) were found to be susceptible to all the antibiotics tested. All the resistant strains were found in the reservoir A. The resistant strains was mainly *Salmonella* Typhimurium and

Salmonella Posoeldorf. Three strains of *Salmonella* were resistant to several antibiotics, including two different families of antibiotics (Table 1).

The strains *S. Poeseldorf* A14-2 and *S. Typhimurium* A2-2 presented resistance exclusively to Gentamicin and Amoxicillin respectively.

Salmonella sp A3-1 showed a double resistance to nalidixic acid and ciprofloxacin, (Table 1). The rest presented multiple antibiotic resistance. Three (60%) of the 5 resistant strains showed a cross resistance to quinolones. The strains *S. Typhimurium* A4-2 was resistant to 4 antibiotics and *S. Poeseldorf* A11-2 resistant to 5 antibiotics. Only two strains, one *S. Typhimurium* (A4-2) and *S. Poeseldorf* (A11-2) were resistant to both penicillin and quinolones. Resistance to Gentamicin (aminoglycoside) was found in 11.12% of isolates. *Salmonella* strains were all sensitive (100%) to 3rd generation cephalosporins (C3G) (Table 2).

3.2 Discussion

Eighteen samples of the 112 samples were positive to *Salmonella* detection (16%). This bacterium was detected more often (38%) by Krzyzanowski [23] in Brazil. Eight different *Salmonella* serotypes have been identified. The number of serotypes found is higher than [24] who found two serotypes in their study of raw sewage in Morocco but lower than the 35 serotypes found in USA [17] and the 19 serotypes found in Czech Republic [25]. This difference could be explained by the number of

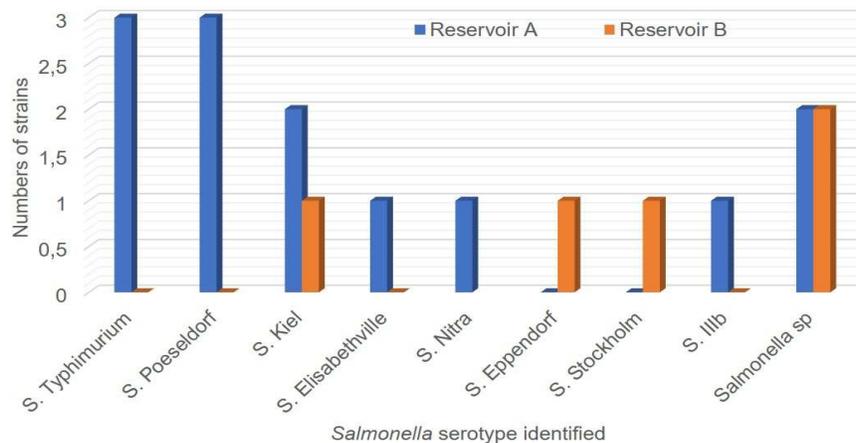


Fig. 4. Distribution of *Salmonella* serotypes according to reservoir

samples analyzed and by the sampling frequency. The difference in serotypes is both quantitative and qualitative. Aboueloufa [24] found mainly strict human *Salmonella* including Typhi and Paratyphi while Benmoussa [26] isolated *Salmonella* Butantan. The presence of *Salmonella* in wastewater is indeed an indicator of biological risk [26] so because this study involves urban wastewater, it was to be expected that the strains of *Salmonella* isolated would coincide with those most frequently isolated from humans over the same geographical area [27]. *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Typhi were the major serotypes isolated from human pathology in Abidjan [28]. The existence of differences between serotypes proceeding from wastewater and from humans in Côte d'Ivoire is a common finding as wastewater can often contain strains of different origins [27, 13]. In Brazil, *S. Typhimurium* was also identified but the most prevalent in wastewater was *S. Infantis* [23]. The presence of *Salmonella* strains of human and animal origin in wastewater might suggest the anthropogenic contribution in wastewater contamination by *Salmonella* as described by Odjadjare and Olaniran [29] and highlighted in Gourou Basin by Akossi [7]. In Sweden, Sahlstrom [4] showed *Salmonella* isolated in sewage sludge traced back to human cases of salmonellosis. Then, *Salmonella* strains responsible for human and animal pathology in wastewater could be used to monitor clones circulating in the populations [30]. Previous research work on *Salmonella* in wastewater in Ivory Coast had not indeed highlight the serotypes identified here [30]. The differences could be due to lack of link between the collectors studied and also by the differences in population and therefore pressure on the various receiving canals as described by Berge et al. [17]. Therefore wastewater could help in the monitoring of salmonellosis on one hand and early detection of their presence on the other [17].

In this study, 27.80% of isolated *Salmonella* were resistant to at least one antibiotic and 16.67% showed cross-resistance to quinolones. Strains exhibited resistance to certain high-level penicillinase (11.11%). The third generation cephalosporin were active on all the *Salmonella* strains tested as observed by Odjadjare and Olaniran [29] in South Africa. The resistance rates of *Salmonella* in water was greater than those found in Morocco where *Salmonella* strains isolated by Benmoussa et al. [26] were

susceptible to beta-lactam and quinolones. The study of antibiotic resistance of *Salmonella* in Ivory Coast has found lower rates of resistance in humans and animals to quinolones (10%) but much higher in face of resistance to penicillins (78%) and cephalosporins (31%). [28]. This high resistance of *Salmonella* to quinolones is again a problem due to the use of these molecules in the treatment of Salmonellosis. Indeed resistance to this family has remained stable or even increased rapidly and some strains are naturally resistant to them [29]. However, the resistance of *Salmonella* to C3G seems to decrease. It was around 18% in the wastewater in the Ivory Coast in 2014 [31], and is rarely reported in recent years [29]. It highlights the importance of a better utilization of C3G, which remains the best treatment. The study of resistance of *Salmonella* to antibiotics showed that all phenotypes cross-resistant to quinolones came from the reservoir A. This could be explained by the reception of sewage of the hospitals in the studies reservoirs. The drainages system that runs through the northern part of Abidjan is emptied into the reservoir A. This receiving canals drained all waste including hospitals effluents. The main teaching hospitals of the city are located in Cocody and the Gourou Basin because of their localization their wastewaters are drained into the reservoir A, which is not the case in the reservoir B. The influence of nearer environment may explain some of the differences observed in the variability of *Salmonella* in the two sites [17]. The presence of *Salmonella* in wastewater and particularly resistant strains could therefore be a potential risk of morbidity among the surrounding population [3] but also of mortality given the limited therapeutic options available [4]. *Salmonella* is easily transmitted by water, and so the emergence of antibiotics resistant or multidrug resistant wastewater may be catastrophic to our country populations. However, contaminated water can also be a major factor in the spread of epidemics [11].

The treatment of wastewater prior to their discharge into the lagoon is therefore necessary [32] and should be treated as urgent in order to protect the populations of *Salmonella* infections and also protect surface and ground water in the region against *Salmonella* contamination, because resistant *Salmonella* isolates could spread over long distances in the environment [4].

Table 1. Antibiotic resistance among Salmonella strains isolated from reservoir A and reservoir B wastewater

Strains	Resistance
Reservoir A	
S. Elisabethville (A2-b)	Susceptible
S. Typhimurium (A7-2a)	Susceptible
S. Kiel (A7-1b)	Susceptible
S. Kiel (A1-1)	Susceptible
S. Kiel (A11-1b)	Susceptible
S. Poeseldorf (A5-2)	Susceptible
S. Nitra (A7-1b)	Susceptible
S. Illb (A12-2)	Susceptible
<i>Salmonella</i> sp (A2-a)	Susceptible
S. Poeseldorf (A14-2)	G
S. Typhimurium (A2-2)	Amx
<i>Salmonella</i> sp (A3-1)	NA, Cip
S. Typhimurium (A4-2)	Amx, Amc, NA, Cip
S. Poeseldorf (A11-2a)	Amx, Amc, NA, Cip, G
Reservoir B	
S. Stockholm (B5-1)	Susceptible
S. Eppendorf (B5-2)	Susceptible
<i>Salmonella</i> sp (B10-1b)	Susceptible
<i>Salmonella</i> sp (B6-2a)	Susceptible

S= *Salmonella* ; AMX=Amoxicillin ; AMC= Amoxicillin+Clavulanic Acid ; CTX= Cefotaxime ; CRO= Ceftriaxone ; NA Nalidixic Acid ; CIP= Ciprofloxacin ; G=Gentamicin

Table 2. Antibiotic resistance profile of Salmonella strains recovered from reservoir A and reservoir B wastewater (n = 18)

	Resistance (%)	Susceptible (%)
Amoxicillin	3 (16,67)	15 (83,33)
Amoxicillin +Clavulanic Acid	2 (11,11)	16 (88,89)
Cefotaxime	0 (0,00)	18 (100)
Ceftriaxone	0 (0,00)	18 (100)
Nalidixic Acid	3 (16,67)	15 (83,33)
Ciprofloxacin	3 (16,67)	15 (83,33)
Gentamicin	2 (11,11)	16 (88,89)

4. CONCLUSION

The Gourou Basin that we studied conveys wastewater contaminated with *Salmonella* resistant to antibiotics. The outcome of our findings highlighted a significant risk of morbidity for people linked to the risk of *Salmonella* infections but also the possibility of therapeutic failure.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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