

ANTIBIOTIC RESISTANCE PROFILE OF *SALMONELLA* SPP ISOLATED FROM THE SEWAGE OF UNIVERSITY CAMPUS OF COCODY, ABIDJAN, CÔTE D'IVOIRE

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ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Background: Recent scientific reports have shown the contamination of wastewater by resistant *salmonella* to antibiotics. Wastewater is often used in Abidjan for agriculture and this practice could be sources of foodborne diseases like salmonellosis. Yet, the knowledge of resistant *salmonella* of wastewater's in Ivory coast is limited

Aims: The objective of this study is to establish the susceptibility profile of *Salmonella* spp. isolated from wastewater to antibiotics and to an antimetabolite commonly used in human medicine.

Study design: Spatio-temporal study

Place and Duration of Study: The study took place from august 2008 to january 2009 at the campus of the Cocody university (Abidjan).

Methodology: Each week, wastewaters were sampled from 4 sewers already identified because of their importance in the study site. *Salmonella* sp were isolated by the classical microbiological method and antibiotics resistances were studied by the method of disk diffusion according Kirby-Bauer. The serotypes of *salmonella* were identified according the Kauffman-White table

Results: Five serotypes of eleven strains, of which 4 Hato, 3 Farmsen, 2 Derby, 1 Essen and 1 Ovonmouth, have been used for the realization of the tests of disks of antibiotics. Among the various classes of antibiotics tested, high resistance was found with sulfonamid (100%) following cefotaxime (46.67%) and tetracycline (9.1%), while ampicillin, amoxicillin + clavulanic acid, gentamicin, kanamycin, amikacin, ciprofloxacin, nalidixic acid and chloramphenicol being the most potent with 100% sensitivity. In generally, the strains have been sensitive (85.94%) but the serotype Derby, Hato and Farmsen were showed some resistance.

Conclusion: There was resistant *Salmonella* in wastewater in cocody. Despite the effectiveness of antibiotics on *salmonella* strains, the level of resistance to the 3rd generation of cephalosporin and to the sulfamid is worrying. The study had to be continued for identify the impact of *salmonella* resistance in wastewater on people.

Keywords: wastewater, Salmonella spp, Resistance, Sensibility, Antibiotics, urban environment

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Due to an uncontrolled galloping ~~demography~~demographic increase, cities in developing countries are experiencing a serious problem of wastewater management and their direct or indirect influence, in a long or short term, on the environment and public health [1]. Abidjan, the Ivorian economic capital and most populous city of francophone West Africa, did hardly escape this issue. Wastewater represents for these cities a resource but also a danger [2]. Directly discharge into the sewerage network and outside [3], without any prior treatment [4] [5], wastewater is used to irrigate crops, despite health risks [6], by disadvantaged populations for which this practice generates substantial financial resources [7]. Wastewater origin, household composition can be extremely variable and depends on several factors, which are the original composition of drinking water, the various uses by individuals who can bring a quasi-infinite number of pollutants, and finally the users themselves who will reject the organic matter (urine, faeces) into sewers [8]. Also, they participate in the contamination of plants, crops while reused in agriculture, or through surface waters in the presence of an important rainfall [9] [10], and groundwater due to their infiltration in the natural receiving environment and accentuated by hydrogeological conditions [11].

~~Diarrhea and food-borne pathogens~~Pathogens for humans and animals ~~both are~~may be excreted ~~in nature~~with the feces of the infected individuals or healthy carriers and ~~generally~~ participate in the final composition in sewage. [2]. ~~In Abidjan, The it has been estimated that the proliferation of sewage deposits in the streets and alleys of disadvantaged suburbs of the neighborhoods, would increase~~the sources of contamination ~~to-for diarrheal enteric diseases~~with an average prevalence of about 19% ~~in disadvantaged suburbs in Abidjan~~[3].

Among the ~~offending pathogenic~~ bacteria, Salmonella ~~Spp~~spp. are responsible for typhoid-like ~~infectious infections or diarrhea diseases~~in human and animals [12] [2].

~~Several studies~~ have correlated the occurrence of ~~salmonella~~Salmonella infection with the use of water contaminated with human ~~or animal~~ feces ~~and or animal~~. [13]. The increase in strains of salmonella involved in outbreaks also ~~highlighted~~highlights the proliferation of antibiotic-resistant serovars. Koivunen's work cited by Berge et al. reported the high prevalence of salmonella in sewage and the risk that it might ~~be represent~~for public health, especially in absence of appropriate treatment, with the emergence of resistant to antibiotics' strains [13]. It is obvious ~~to the authors~~that the water would play an important role in the spread of Salmonella strains in the environment but also in the human species.

Studies have shown that the wild-type strain of Salmonella is characterized by its sensitivity to all ~~active antibiotics~~antimicrobials ~~active against on~~enterobacteria [14] [15]. However, there are concerns about progressive ~~increase in the~~resistance pattern ~~or even~~ environmental clinical strains, given the ~~misuse of antibiotics in human medicine and in animal husbandry~~ [16].

The overall objective of this study is to establish the ~~antimicrobial~~ susceptibility profile of Salmonella spp. isolated from wastewater sewage ~~of the Cocody University campus in Abidjan, Côte d'Ivoire~~. ~~to antibiotics and to an antimetabolite~~antimicrobials commonly used in human medicine. ~~The study was conducted at Cocody University campus in Abidjan, Côte d'Ivoire. Sampling was performed in the sewage channels which flow directly into an opencast of the communal sewerage network, dragging in the Ebrie lagoon without any prior treatment. The Cocody~~

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Comment [V2]: You mean the sewers? This sentence is too long too. Please split it in two sentences and write them more directly. For instance:
Wastewater reused in agriculture may be responsible for the contamination of plants an crops, Moreover, rainfalls and soil infiltration may lead to polution of surface and ground water with sewage.

Comment [V3]: It Increases by 19% or the prevalence is 19%?

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campus is surrounded by many precarious neighborhoods namely: WASSA, CHU village, CHU Baghdad to mention some.

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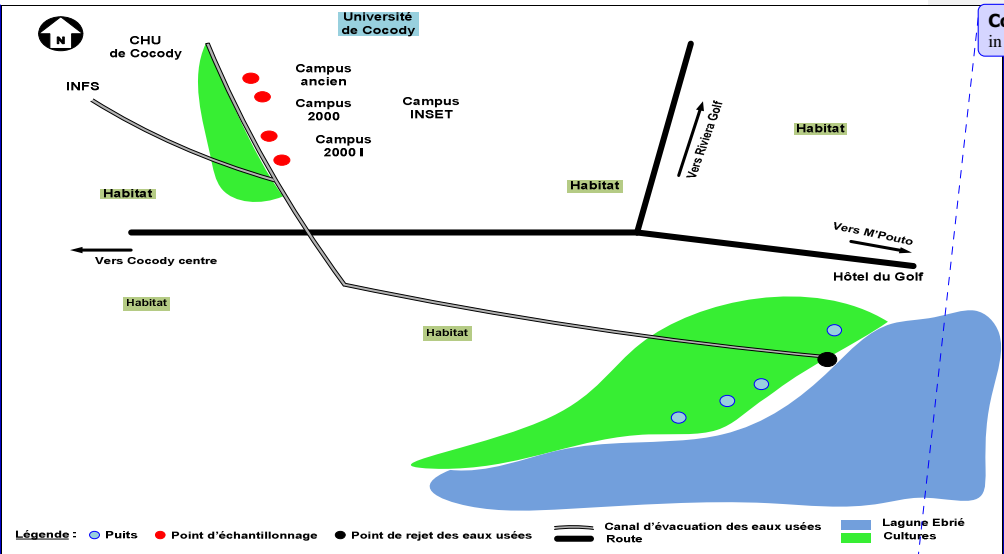
2. MATERIAL AND METHODS
2.1. Study location

Located in the Cocody's communal area, the University campus houses at least 8,000 inhabitants not to mention the thousands of people crossing in the area. Moreover, the Cocody campus is surrounded by many precarious neighborhoods such as WASSA, CHU village, CHU Baghdad. Wastewaters from toilets, laundries, household's generated wastes are rejected by sewers into a communal open-air collector that carry them in the Ebrié lagoon (Figure 1), side of the five stars "Golf Hotel". This spatio-temporal study Sampling were conducted, began infrom August 2008 and ended into January 2009. The sampling sites figure here after.

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Figure 1: Map depicting the Cocody's communal area, Sampling sites' location and the discharge channel of wastewater into the lagoon and the sampling sites of the study (Source: André GADJI, 2009)

2.2. Sampling procedure

4-Four sewers were selected for samples uptake the samplings, based on the following criteria: sewers capacity to drain a significant flow of wastewater, safe accessibility, and their physical state (sewer in good condition). The samples were collected using a sterile jar and packaged in 0,5 Liter sterile polystyrene bottles. The levies were made every morning between 7 a.m. and 8 p.m., once per a week for ten weeks.

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After collection, samples have been labeled (date and time of sampling, location, batch number), sent to the laboratory in a cooler containing a cold accumulator and analyzed

immediately. A total of 40 samples were collected from four (4) different sewers. Each output sample consisted of 4 sub-samples (2 L of wastewater) per sewer.

2.3. Detection of Salmonella in sewage wastewater

The detection of Salmonella spp. in sewage was made according to the method defined by Edel and Kampelmacher [17] and retain by the international standards [18]. This classical method involves 4 steps namely, non-selective pre-enrichment, enrichment in selective liquid, the solid selective medium isolation and biochemical and serological identification.

2.3.1 Preenrichment in liquid medium

10 mL of wastewater were diluted in 90 ml of buffered peptone water. The resultant suspension is mixed for about 3 min. A control suspension (containing only peptone water) was also performed. All these suspensions were incubated in an oven at 37 °C for at least 16 hours and 20 hours in sterile plastic pots.

2.3.2 Enrichment in selective liquid media

From each parent and control suspensions, a sterile tubes of Rappaport Vassiliadis media (RV10) was seeded. 0.1 mL of each subculture (suspension and control) has been added to 10 mL of RV10 and incubated at 42-44 °C for 20 hours in the oven.

2.3.3 Culture on selective isolation media

Hektoen Agar in Petri dishes was seeded using a sterile Pasteur pipette by streaks of exhaustion from the two selective liquid media of a same parent suspension. The plates were then incubated at 37 °C for 24 hours. After 24 hours of incubation, 3 to 5 typical colonies by Petri dish were selected for the stages of morphological and biochemical identification.

All typical colonies of Salmonella have been subjected to the tests of slide agglutination using agglutinating serums Kauffmann-White scheme and completed by Le Minor identification process [19]. This step allowed to clarify the antigenic structure of each colony and to confirm biochemical identification.

Serotyped strains were assessed for their antibiotic resistance's pattern.

2.4. Evaluation of the antibioresistance [20].

The agar diffusion method according to Kirby-Bauer was used for this study. Eleven (11) antimicrobial chosen among five (5) different families were tested namely ampicillin, amoxicillin + clavulanic acid, cefotaxime, gentamicin, kanamycin, amikacin, ciprofloxacin, nalidixic acid, chloramphenicol and sulfamide which is an antimetabolite.

2.4.1 Inoculum preparation

Using Pasteur pipette one to two colonies were taken from a 24 hours' culture on regular agar and transferred in a 2 mL saline suspension (0.9% NaCl) in a bottle. The solution was homogenized with a VORTEX. Solution density was measured with a DENSIMAT to read the turbidity. 100 µL of the solution is diluted into 10 mL of saline water to adjust to McFarland 0.5 standard (~ 10⁸ cfu/ml).

2.4.2 Seeding

A small amount of the inoculum is flooded on to a Mueller-Hinton agar previously cast in a 90 mm diameter plate. The surplus of the inoculum is removed and antibiotic discs are then placed on the surface of the agar with pliers or an automatic applicator. Boxes rest for 3 to 5 min to allow the dissemination of antibiotics into the agar before incubation in an oven at 37 °C for 18-24 hours.

2.4.3 Reading

The Diameter of inhibition zones after 24 hours incubation at 37 °C is measured using a foot slide for each disk and the values interpreted as (sensitive (S), resistant (R) or intermediate (I) according to the CA-SFM instructions.

3. RESULTS

3.1. Proportion of Salmonella within the overall sampling

Out of the 40 sewage samples analyzed, the Salmonella rate is estimated as high as 42.5% (fig.2). In decreasing order, Species ranged from 36.36% Salmonella Hato, Salmonella Farmsen with 27.27%, Salmonella Derby with 18.18%, Salmonella Essen and Ovonmouth with 9.09% each (fig. 3).

The 42.5% rate reflects the impact of this organism on the urban environment and reveals the poor quality of these waters discharged directly into the lagoon without any treatment.

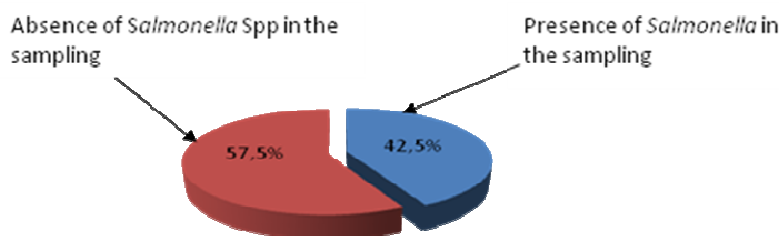


Figure 2: *Salmonella* isolates rate in the sewage wastewater dans

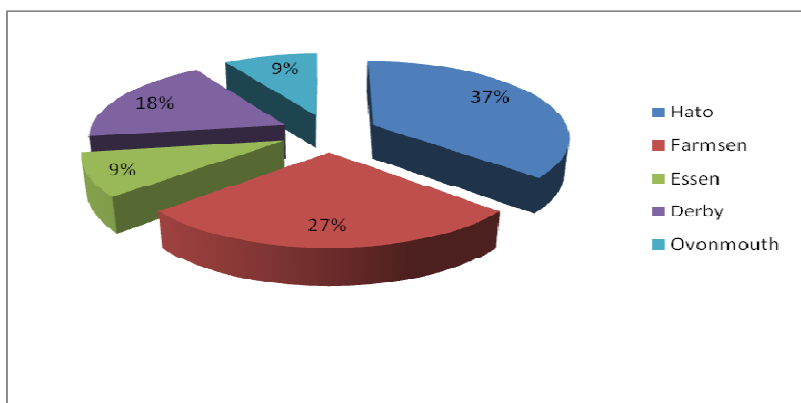


Figure 3 : *Salmonella* Strains' identified in the sampling

3.2. *Salmonella*'s strains susceptibility vis-à-vis the antibiotics and the antimetabolite

Salmonella's resistance and sensitivity profile to antibiotics was assessed for the 11 serovars of *Salmonella* including 4 Hato, 3 Farmsen, 2 Derby, 1 Essen and 1 Ovonmouth. All Strains were resistant to sulfamide (antimetabolite), 2 were resistant to cefotaxime, three (03) presented an intermediaries profile and finally a single strain has resisted to tetracycline. The overall result reveals that 85.94 percent of tested strains were sensitive to antibiotics, 11.58% resistant and 2.48% of intermediaries profiles. (Table I)

Table I: Distribution of Sensitive, intermediaries and resistant strains per antimicrobial drugs

Antibiotics/antimetabolite	Sensitive N=11	Intermediary N=11	Resistants N=11
AM	11	00	00
AMC	11	00	00
CTX	06	03	02
NA	11	00	00
GM	11	00	00
K	11	00	00
AN	11	00	00
CIP	11	00	00
C	11	00	00
TET	10	00	01
Antimétabolite			
SSS	00	00	11
Percent (%)	85.94	02.48	11.58

β lactam : ampicillin (AM), amoxicillin + clavulanic acid (AMC) & céfotaxim (CTX) ;

Aminosid : amikacin (NA), gentamicin (GM) et kanamycin (K) ;
 Quinolones : Nalidixic acid (AN) et ciprofloxacin (CIP) ; phenicol : chloramphenicol (C) ; cycline : tetracycline (TET) ;
 sulfamides (antimetabolite) (SSS).

Among the Salmonella strains observed, Salmonella Derby has most resisted to cefotaxime and tetracycline with a resistance level of almost 50% (table II). Its overall resistance pattern to antibiotics and the antimetabolite is estimated at 18.18%. Salmonella Hato is the 2nd strain which resisted to cefotaxime at a rate of 25% of resistance and a level of overall resistance of 11.36%. Salmonella Farmsen and Ovonmouth had the same level of resistance but of different sensitivity, i.e. 87,88% and 81.82% respectively. On the other hand, all serovars resisted the only antimetabolite (sulfamide) commonly used in human medicine. Intermediate pattern in the presence of cefotaxime were also observed. These cefotaxime resistant strains were from Ovonmouth, Hato and Farmsen species.

Table II: Salmonella strains' antibioresistance profile

	S. Hato			S. Farmsen			S. Derby			S. Essen			S. Ovonmouth		
Antibiotiques	% S	% I	% R	%S	%I	%R	%S	%I	%R	%S	%I	%R	%S	%I	%R
AM	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
AMC	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
CTX	50	25	25	66,67	33,33	0	50	0	50	100	0	0	0	100	0
NA	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
GM	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
K	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
AN	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
CIP	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
C	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
TET	100	0	0	100	0	0	50	0	50	100	0	0	100	0	0
Antimétabolite															
SSS	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Global resistance	86,36	2,27	11,36	87,88	3,03	9,09	81,82	0	18,18	90,9	0	9,1	81,82	9,09	9,09

S : sensitive ; I : intermediate ; R : resistant

3.3. Compared effectiveness of antibiotics and of an antimetabolite vis-à-vis of Salmonella strains

Susceptibility testing of Salmonella Strains vis à vis several antibiotics and antimetabolite revealed the ineffectiveness of two antibiotics against some strains of Salmonella such as Derby and Hato. Cefotaxime, a 3rd generation β -lactam cephalosporin, has been less

effective among the tested antibiotics. His efficiency rate is estimated less than 50%. Tetracycline is the second molecule to which a strain of Salmonella has resisted with an efficiency rate of approximately 91%. Other antibiotics i.e. ampicillin, amoxicillin + clavulanic acid, the gentamicin, kanamycin, amikacin, ciprofloxacin, nalidixic acid and chloramphenicol were effective against Salmonella strains after 24 hours of incubation at 37 ° C.

Conversely, the least effective antibiotic (cefotaxime) in our study compared to the sulfonamide is significantly higher than this antimetabolite in terms of effectiveness against the strains of Salmonella.

4. DISCUSSIONS

The strong presence of Salmonella in domestic wastewater, estimated 42.5% provides information on the potential impact of microbial pollution of domestic effluent on population's health and on the environment. This frequency of Salmonella may be due to the high population's density on the Cocody campus where sanitation and hygiene conditions were precarious during the study period.

The massive presence of Salmonella spp. in wastewater is common to many big city but especially noted at the level of treatment center in Europ. [21][10][13][2]

This bacterium is primarily of intestinal origin and found normally in human sewage, its regularity in the outside environment could pose a public health problem. In Côte d'Ivoire, Dongo and al. [3], noted adverse populations behavior rejecting their sewage into the open sewers and or on the street. The risk for health is related to the rejection of these effluents in the environment without any treatment, to their infiltration in groundwater from favorable hydrogeological conditions [11] and their use or re-use in market gardening [22].

The majority of the 2400 Salmonella serovars should be considered potentially pathogenic. Studies on a collection of fresh vegetables from the field [23] and on the waters of sprinkling vegetables collected, in three communal markets of Port-Bouet, Koumassi and Cocody all located in Abidjan [24], showed a presence of Salmonella in the waters of vegetables.

Hence, one of the major issue remains that 44% of households in Abidjan does not disinfect vegetables before consumption, according to the behavioral study by Lorougnon [25].

The vast majority of Salmonella present in the environment (land, water, plant) comes from fecal contamination [26]. This fact should therefore impose a rigorous and efficient domestic wastewater management and adequate treatment prior to discharge into the environment. The proliferation of wild deposits of sewage in the streets and alleys of the neighborhood would increase sources of contamination to diarrheal diseases with an average prevalence of about 19% in disadvantaged suburbs in Abidjan [3]. This would also favor the emergence of Salmonella infections.

Salmonella sp would be indeed at the origin of approximately 48% of foodborne diseases in Côte d'Ivoire [27] and also affects animals. If it is established a positive correlation between human salmonellosis and animal origin, the presence of Salmonella strains responsible for human infection in wastewater is worrying. Indeed, the serotypes of Salmonella Essen and Farsem identified in this study have already been cited in human pathology in Côte d'Ivoire; Essen and Derby serotype in veterinary medicine. In the light of this, humans, poultry and

wastewater have in common in Côte d'Ivoire. *Essen of Salmonella serotype without excluding others.*

It is proved that wild serotype is characterized by sensitivity to all active antibiotics on enterobacteria [15], however, resistant strains are definitely emerging.

In this study, antibiotic susceptibility testing by the conventional method of disks revealed that studied serovars are mostly sensitive to all commonly used antibiotics in human medicine, including cyclin, phenicols, quinolone, aminoglycosides and β -lactam except the 3rd generation. In fact, the study of [14] showed that *Salmonella* spp. is sensitive to most broad-spectrum antibiotics. However, resistances have been observed for cyclin notably tetracycline and for 3rd generation cephalosporin, whose resistance pattern range respectively at 9.1% and 18.2% on a total of 11 strains. It also noted 14,16% global resistance with 11.58% resistant and 2.48% of intermediaries profiles. This level of resistance is near of Oman's level [28] but lower than that found by Melloul [21] in Morocco who isolated 36.5% resistant strains of salmonella in wastewater perhaps because of the little numbers samples we got. All salmonella strains were resistant to sulfonamide (100%). Prior studies [29] and those conducted by Bonny [30] have highlighted the Sulfonamide and tetracycline resistance towards non typhoid strains. Respectively, the sulfonamide resistance rate of 11.59% and 93.75% were reported versus 4.35% and 56.25% for the tetracycline.

The presence of *Salmonella* spp. of resistant pattern in wastewater in Côte d'Ivoire is disturbing in light of the increasing rate observed in clinical and veterinary. According to Coulibaly and al, the antibiotic resistance rates of *Salmonella* spp. increased from 0% in 1980 to 10.52% for the Fluoroquinolone and to 31.57% for the third generation cephalosporin (C3G) in 2009 in humans. [27].

The strains of animal origin were resistant to tetracycline close to 100% and to 6.82% for the third generation cephalosporin in 2009 in poultry.

Therefore, the resistance of *Salmonella* strains isolated from wastewater, against the third generation cephalosporin seems to be greater than those observed in humans and animals even if it is not statically proven.

Avian strains resistance to the tetracycline is much higher than what is observed in this study and in the literature. The low quantity of sample could explain this situation

Cross transmission of *Salmonella* strains resistant to the antibiotic from human in the animal and vice-versa is no longer a subject of debate, and both live in the same environment which they contaminate. [31]

The presence of tetracycline resistance gene in *Salmonella* spp isolated from untreated sewage wastewater was described by Zhang et al [32]. Thus affirming the role that could play the wastewater in the transfer of resistance gene in *Salmonella* species. [32]. Wastewater could therefore play a fairly important role in the re-circulation of resistant strains of salmonella SP. in human and animal populations.

This relatively high rate of resistance of *Salmonella* sp. in the C3G as well in humans, in animals and in the environment in Côte d'Ivoire must be closely monitored especially when this molecule represent the basis of the treatment of salmonellosis especially in children [13]. The decrease in sensitivity to the C3G was already 8.1% in Pediatrics in 2009 [33]. WHO noted the alarming increase in the incidence of antibiotic-resistant *Salmonella* strains which would be due to the misuse of these antibiotics both in human medicine and in animal

husbandry [16]. This could be responsible too for the presence of resistant salmonella strain in wastewater [34]. The resistance genes of antibiotics are transferable between strains in the environment, another worrying reason [32] [35]. Courvalin et al. (1985) believe that the bacterial antibio-resistance is based on a modification of the genetic capital that allows a strain to grow in the presence of an antibiotic concentration higher than the concentration that inhibits the majority of strains of the same species [36]. This observation can be added, depending on what is allowed [15], specifically the resistance of *Salmonella* spp. to antibiotics relies on the following assumptions:

- IES strains not subject to selection pressure, remain sensitive to antibiotics;
- There is an increase in the levels of resistance for some serovars such as *Salmonella* Typhimurium, *Salmonella* Hadar and *Salmonella* Saint Paul in relation to livestock husbandry practices;
- There is an increase in the acquisition of multi-drug resistance;
- There is an increase in the number of determinants of resistance integrated into the chromosome;
- the use of new molecules of antibiotics in livestock frequently increases the level of resistance against these molecules.

Of the foregoing, it is feared a progressive environmental strains resistance, but also clinical resistance [37] as the water environment contains residues of antibiotics [38] that could contribute strongly to the maintenance, the emergence and spread of bacterial populations exhibiting a low level of resistance and ready to evolve towards the resistance [39].

However, although resistant strains were observed, the study showed a high level of sensitivity of all serovars to eight antibiotics across a broad spectrum. These are the antibiotics from phenicoles family (chloramphenicol), aminoglycosides (amikacin, gentamicin and kanamycin), quinolones (nalidixic acid and ciprofloxacin) and a β -lactam particularly of the Penicillins group has (ampicillin and amoxicillin + clavulanic acid). They will certainly be the antibiotics of choice in the case of salmonellosis.

5. CONCLUSION

The study highlighted, the presence of various *Salmonella* serotypes in sewage wastewater, in that case *Salmonella* Derby, *S. Essen*, *S. Farmsen*, *S. Hato* and *S. Ovonmouth*. It has also highlighted the levels of resistance of these strains to commonly used antibiotics. Thus our study revealed that the strains are resistant to 3rd generation cephalosporins β -lactam antibiotics (cefotaxime), to cyclin including tetracycline and antimetabolites such as sulfonamide. However, despite the 11.58% overall resistance, *Salmonella* serovars remain totally sensitive to the phenicoles, aminoglycosides, quinolones and other β -lactam particularly Penicillins Group A. The phenotypic characteristics of *Salmonella* underscore the challenges related to the treatment of salmonellosis infection and the rational use of antibiotics.

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COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

'Author A': have made the serotyping and antibiotic sensibility test of the strains. he also write the article, prepared the media and identified strains.

'Author B': write the protocol and has took samples in the sewers before made the culture. He identified salmonella with the 'author A'

'Authors C and D': rewrite and correct the article in english

'Author E': designed the study and corrected the protocol

'Author F': signed the protocol, gave the material and supervised all the work with the 'author E'

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