

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

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ABSTRACT

Background: Recent studies have shown that wastewater is contaminated by salmonella sp., a pathogenic antibiotics-resisting bacteria. Using wastewater for irrigation in the city of Abidjan seems to be the source of food-borne diseases such as salmonellosis. However, what we know about these resistant salmonella spp. in wastewater is limited in the country.

Aims: This study aims to establish the susceptibility profile of Salmonella spp., isolated from wastewater to antibiotics and to antimetabolite commonly used by medical practitioners.

Study design: Spatio-temporal variation was taken into account.

Place and Duration of Study: The study took place from August 2008 to January 2009 at the main campus of the university of Cocody in Abidjan.

Methodology: Sampling was done on a weekly basis. Wastewater samples were collected at four different sewers in the campus area. Salmonella sp was isolated by a standard method of laboratory. The resistance of these isolated species to antibiotics was determined according to the disk diffusion method of Kirby-Bauer. The serotypes of salmonella were identified with the Kauffman-White table

Results: Five serotypes of eleven strains, which consist of 4 Hato, 3 Farmsen, 2 Derby, 1 Essen and 1 Ovonmouth, were isolated and tested in order to determine their resistance to antibiotics. Amongst the various classes of antibiotics, high resistance was found to sulfonamid (100%), followed by cefotaxime (46.67%) and tetracycline (9.1%). Ampicillin, amoxicillin & clavulanic acid, gentamicin, kanamycin, amikacin, ciprofloxacin, nalidixic acid and chloramphenicol had a high potentiality: their efficacy in the elimination of the Salmonella sp was proved at a level of 100%. Although the majority of strains tested (85.94%) were eliminated by the antibiotics, the serotypes Derby, Hato and Farmsen showed resistance.

Conclusion: The Wastewater in the area of the main campus of the University of Cocody contains the antibiotic-resisting strains of salmonella sp. In spite of the fact that the efficacy of some antibiotics in the elimination of Salmonella sp. is proved, the resistance of these strains to third generation of cephalosporin and sulfamid is worrisome. Further studies should be carried out to determine the effects of this antibiotic-resisting salmonella species on human health.

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

1. INTRODUCTION

Developing countries are facing serious problems in wastewater management due to demographic explosion and a poor urban planning. This lack of good waste management system has negative influences on the environment and public health [1]. Abidjan, the Ivorian economic capital, and the most populous city of the West African French-speaking countries is not spared from this ordeal. Wastewater which is used by people as a resource in these cities also constitutes a great threat [2]. Directly poured into the sewerage network and in the nature [3], without being treated before [4] [5], wastewater is used to irrigate crops, even though this is risky for health [6], of poor people for whom this practice is certainly a source of making money [7]. The composition of contaminants in wastewater depends on households' drinking water source and domestic water-use; Using these waters can bring a quasi-infinite number of pollutants essentially the organic matters (urine, faeces) in sewers [8]. Re-using water in agriculture is probably the cause of contamination of plants and crops [9]. However, infiltrating rainwater and cesspool leakage may lead to groundwater contamination [10][11].

Human and animal pathogens, excreted by individuals infected or healthy carriers are also constituents of wastewater in the sewage systems [2]. In Abidjan, it has been reported that cesspool leakages within precarious settlements is responsible for the prevalence of enteric diseases (19%)[3].

Salmonella spp. is responsible for ailments such as typhoid-fever infections or diarrhea in human and animals [12] [2]. Several studies have linked the occurrence of *Salmonella* infection to the using of contaminated waters by human or animal faeces [8][10] [13]. Berge et al, reported that the abundance of *salmonella* in sewages, if untreated before re-using is a great health risk, especially with the emergence of strains of *salmonella* sp resistant to antibiotics [13]. Therefore, wastewater is an effective transport medium in the spread of *Salmonella* sp in the environment and amongst humans. Studies have shown that strains of *Salmonella* sp. referred to as wild-type are characterized by their sensitivity to all antimicrobials active against enterobacteria [14] [15]. However, there are concerns about progressive increase in the natural environment of the resistant *salmonella* strains and also due in the presence of resistant clinical strains. The abuse of antibiotics by humans and in animal husbandry could be responsible. [16].

The main objective of this study is to establish the antimicrobial susceptibility profile of *Salmonella* spp. isolated from wastewater sewage of the campus of the University of Cocody in Abidjan (Côte d'Ivoire) to antimicrobials commonly used in medicine to antimicrobials commonly used in human medicine.

2. MATERIAL AND METHODS

2.1. Study location

The campus of the University of Cocody is situated in the commune of Cocody. 8,000 inhabitants and thousands of visitors are estimated to access the campus area daily. The

campus is surrounded by neighboring precarious settlements such as WASSA, CHU village and CHU Baghdad from which households' wastes and wastewater are directly poured into the Ebrié lagoon (Figure 1). Sampling was conducted from August 2008 to January 2009.

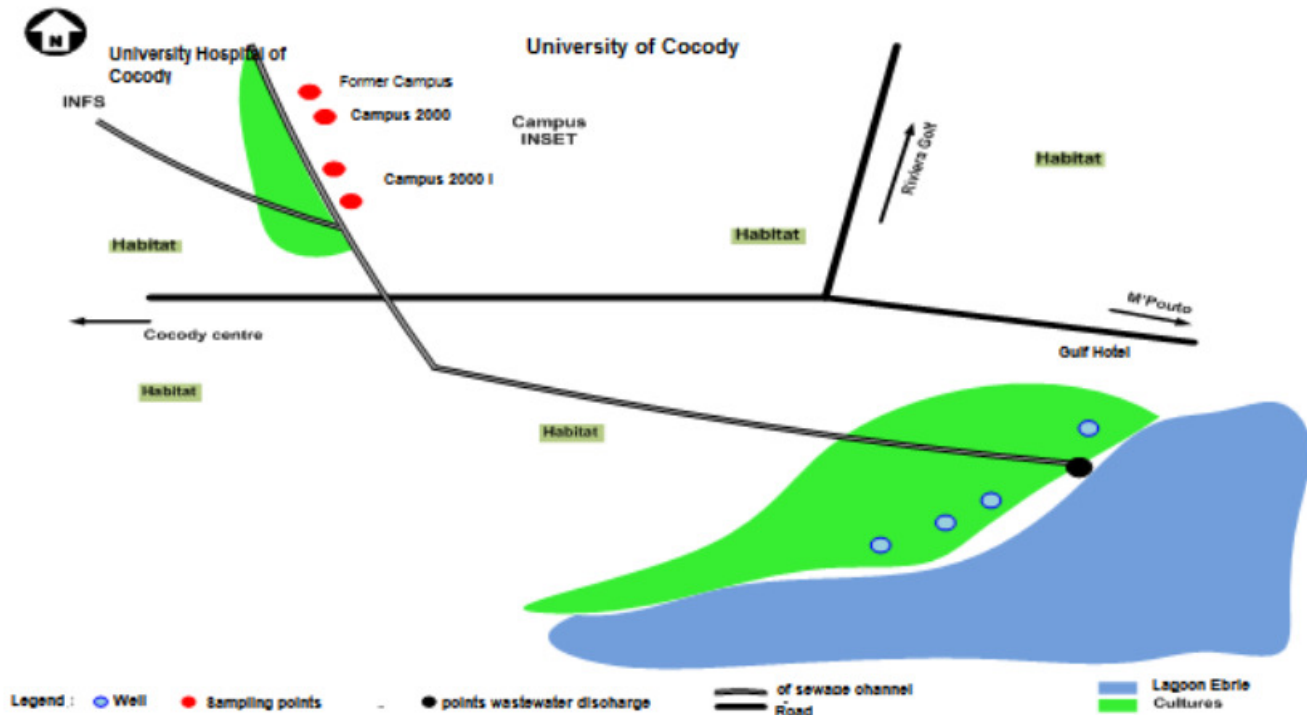


Figure 1: Map depicting the Cocody's communal area, the discharge channel of wastewater into the lagoon and the sampling sites of the study (Source: André GADJI, 2009)

2.2. Sampling methodology

Sampling was carried out in four sewers. The choice of the site was based on the following criteria: sewers' draining capacity, accessibility, and their physical condition. Wastewater samples were collected onto a sterile jar and packaged in 0.5 Liter sterile polystyrene bottles. Collection was done between 7 am. and 8 am., once a week for a period of ten weeks.

Samples were labeled (date and time of sampling, location, batch number), transported to the laboratory in an ice-packed cooler and immediately analyzed. A total of 40 samples were collected from four (4) different sewers. 4 subsamples (2 L of wastewater) per sewer.

2.3. Detection of Salmonella in sewage wastewater

87 The detection of Salmonella spp. was according to the conventional method of Edel and
88 Kampelmacher [17] and retain by the international standards [18]. This involves 4 steps:
89 non-selective pre-enrichment, enrichment in selective media, the solid selective medium
90 isolation and biochemical and serological identification.

91

92 **2.3.1 Pre-enrichment in liquid media**

93 10 mL sample was diluted by 90 ml of buffered peptone solution. The resulting mixture is left
94 to stand for about 3 min. A control (containing only peptone solution) was also run
95 simultaneously. They were subsequently incubated in an oven at 37 ° C for at least 16
96 hours in sterile plastics.

97

98 **2.3.2 Enrichment in selective liquid media**

99 0.1mL subsample is then collected and poured onto sterile tubes of 10mL of Rappaport
100 Vassiliadis media (RV10) and incubated at 42-44 ° C for 20 hours.

101

102 **2.3.3 Culture on selective isolation media**

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

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

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

113

114 **2.4. Evaluation of the antibioresistance [20].**

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

119

120 **2.4.1 Inoculum preparation**

121 Using Pasteur pipette one to two colonies were taken from a 24 hours' culture on regular
122 agar and transferred in a 2 mL saline suspension (0.9% NaCl) in a bottle. The solution was

123 homogenized with a VORTEX. Solution density was measured with a DENSIMAT to read
124 the turbidity. An amount of 100 μ L of the solution is diluted into 10 mL of saline water to
125 adjust to McFarland 0.5 standard ($\sim 10^8$ cfu/ml).

126

127 **2.4.2 Seeding**

128 A small amount of the inoculums was flooded on to a Mueller-Hinton agar previously casted
129 in a 90 mm diameter plate. The surplus of the inoculums was removed and antibiotic disks
130 are then placed on the surface of the agar with pliers or an automatic applicator. Boxes were
131 allowed to rest for 3 to 5 min to allow the dissemination of antibiotics into the agar before
132 incubation in an oven at 37 °C for 18-24 hours.

133

134 **2.4.3 Reading**

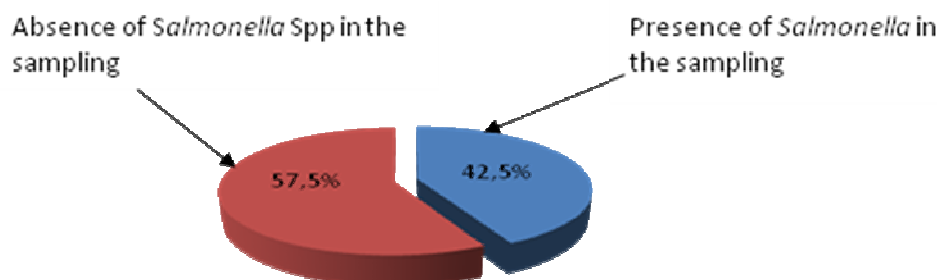
135 The Diameter of inhibition zones after 24 hours incubation at 37 °C is measured using a
136 foot slide for each disk and the values interpreted as (sensitive (S), resistant (R) or
137 intermediate (I) according to the CA-SFM instructions.

138

139 **3. RESULTS**

140 **3.1. The distribution of *Salmonella* sp in the water samples**

141 42.5% of the analyzed samples contained the salmonella sp. 36.36% salmonella Hato,
142 27.27% Farmsen, 18.18% Derby and 9.09% each of Essen and Ovonmouth, in order of
143 decreasing abundance. (Fig.3) This show the poor quality of these domestic effluents
144 directly discharged into the lagoon environments without any form of
145 treatment.



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

147

148

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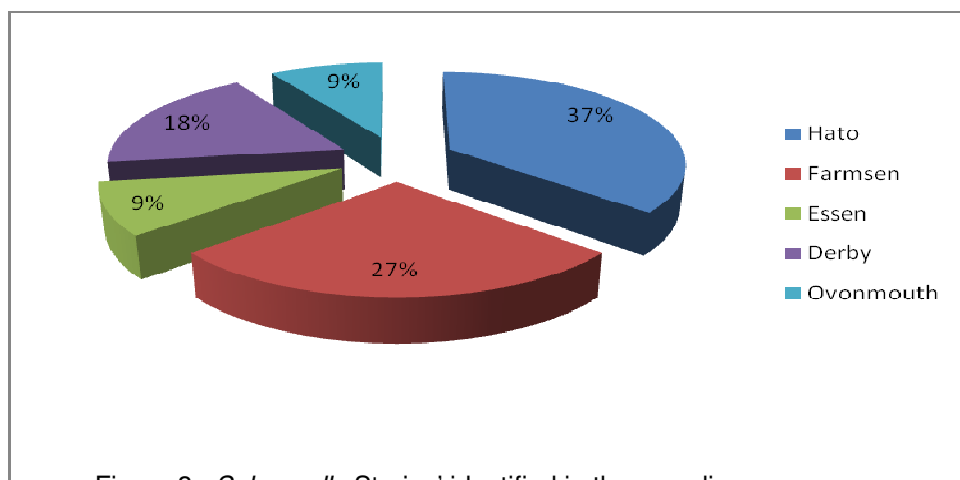


Figure 3 : *Salmonella* Strains' identified in the sampling

3.2. The reaction of salmonella strains to antibiotics and antimetabolite

The resistance and sensitivity to antibiotics by 11 serovars of salmonella sp was assessed. These are 4 salmonella Hato, 3 Salmonella Farmsen, 2 salmonella Derby, 1 Essen, 1 Ovonmouth. All bacteria strains showed resistance to sulfamide (antimetabolite).

Two of them showed strong resistance to cefotaxime, three showed mild resistance to this antibiotic and a single strain was resistant to tetracycline. In general, 85.54% of tested strains were sensitive to antibiotics drug, 11.58% were resistant and 2.48% showed only mild resistance. (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
Antim��tabolite			
SSS	00	00	11
Percent (%)	85.94	02.48	11.58

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

Aminosid : amikacin (NA), gentamicin (GM) et kanamycin (K) ;

Quinolones : Nalidixic acid (AN) et ciprofloxacine (CIP) ; phenicol : chloramphenicol (C) ; cycline : tetracycline (TET) ;

sulfamides (antimetabolite) (SSS).

165

166 Amongst the salmonella strains, Salmonella Derby showed a 50% resistant to cefotaxime
 167 and tetracycline (Table II). The overall resistance to antibiotics is estimated at 18.18%. This
 168 is followed by Salmonella Hato which showed a 25% resistance to cefotaxime and 11.36%
 169 resistance to antibiotics drugs. Salmonella Farmsen and Ovonmouth showed the same level
 170 of resistance but different sensibility (87.88% and 81.82% respectively). All serovars showed
 171 resistance to sulfamide the only antimetabolite used in human medicine.

172 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

173 S : sensitive ; I : intermediate ; R : resistant

174

175 3.3. The effectiveness of antibiotics and antimetabolite in the elimination of 176 the salmonella strains

177 Result reveals that several antibiotics and antimetabolite were ineffective against some
 178 strains of Salmonella such Derby and Hato. Cefotaxime a third generation beta lactam
 179 Cephalosporin was the least effective (50%) in their elimination. Secondly, Tetracycline with
 180 91% efficiency was resisted by some strains. Other antibiotics such as ampicillin, amoxicillin
 181 and clavulanic acid, gentamicin, kanamycin, amikacin, ciprofloxacin, nalidixic acid and
 182 chloramphenical were effective against Salmonella strain after 24 hours of incubation at
 183 37°C. However, the least effective antibiotics (cefotaxime) as proven in this study, had a
 184 significantly higher efficacy in the elimination of the Salmonella bacteria than the metabolite.

185

186 4. DISCUSSIONS

187 The high abundance of *Salmonella* (42.5%) in domestic source wastewater implies a high
 188 exposure risk and environmental pollution. Their abundance in the campus area is due to the

189 density of the population and also to the prevalence of unhygienic and poor sanitary
190 conditions.

191 The occurrence of *Salmonella* sp in wastewater is common issue in many cities. In Europe, it
192 is formal at wastewater treatment and discharge points [21][10][13][2]

193 *Salmonella* sp is usually of intestinal origin and found in human excreta. Its occurrence in the
194 environment lead to great health risks. In Cote d'Ivoire, Dongo et al. [3] reported on the
195 uncontrolled discharge of domestic effluents on the streets and in open sewers. The risk
196 linked to these irrational behaviours comes from their using or re-using in peri-urban area
197 agriculture [22] and their possible infiltration into the subsurface waters under
198 hydrogeological condition [11].

199 The already identified 2400 *Salmonella* serovars worldwide are considered pathogenic.
200 Studies in Abidjan have shown the occurrence of *Salmonella* sp in vegetables [23] and in
201 water used for irrigation [24]. Fresh vegetables collected from three different markets within
202 the commune of Port-Bouet, Koumassi and Cocody has also shown the presence of
203 *Salmonella*. According to Lorougnon [25], this is worrisome since 44% of households in the
204 city don't disinfect vegetables before consomation.

205 The greatest part of *Salmonella* present in the environment (soil, water, plant) comes from
206 animal and human excreta [26]. It is therefore necessary that domestic effluents have to be
207 treated before discharging them into the environment. The uncontrolled disposal of effluents
208 is responsible for the prevalence of diarrhea (19%) within the precarious settlements in
209 Abidjan [3]. This encouraged the growth of *Salmonella*.

210 *Salmonella* sp. is at the origin of approximately 48% of food-borne diseases in Côte d'Ivoire
211 [27] both in human and in animals. It is prouved that *salmonella* of human origin and
212 *salmonella* of animal origin are the same.. Therefore, the presence of *Salmonella* strains
213 responsible for human infection in wastewater is worrisome. In fact, the serotypes Essen and
214 Farsem identified in this study have already been cited in human pathology in Côte d'Ivoire
215 while the Essen and Derby serotypes have been cited in veterinary medicine. So it can be
216 concluded that in Côte d'Ivoire, the Essen serotype can be found in humans, animals and
217 wastewater, without excluding the other serotypes.

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

220 This study shows that the tested serovars are sensitive to all antibiotic drugs commonly used
221 in human medicine. These drugs include cyclin, phenicoles, quinolone, aminoglycosides and
222 β -lactam; except the 3rd generation of cephalosporin. In fact, the study of Pilet et al. [14]
223 showed that *Salmonella* spp. is sensitive to most of the broad-spectrum antibiotics.
224 Nevertheless, it has been observed that there was a resistance to cyclin particularly
225 tetracycline and also the 3rd generation cephalosporin, the resistance pattern of which
226 ranged between 9.1% and 18.2% respectively. Generally, there was about 14.16% of
227 *salmonella* bacteria that have shown a resistance to all the antibiotic drugs tested; 11.58%
228 were strongly resistant while, 2.48% had an intermediary resistance. This degree of
229 resistance is similar to the findings of Al-Bahry et al. in Oman[28] but, lower than that of
230 Melloul et al [21] in Morocco; who isolated 36.5% resisting strains of *salmonella* in
231 wastewater. All the tested *salmonella* strains were resistant to sulfonamide (100%). Previous
232 studies [29] and those realised by Bonny in Côte d'Ivoire [30] have obviously demonstrated
233 the resistance of non-typhoid *salmonella* strains to sulfonamide and tetracycline. The

percentage of resistance to sulfonamide ranged between 11.59% and 93.75% while, the resistance to tetracycline ranged between 4.35% and 56.25%.

The presence of antibiotic-resisting strains of *salmonella* spp. in wastewater in Côte d'Ivoire is disturbing because of the increasing rate of salmonellosis observed in clinics and by veterinaries. According to Coulibaly et al, the percentage of resistance of *salmonella* sp to Fluoroquinolone increased from 0% in 1980 to 10.52% and to 31.57% to the third generation cephalosporin (C3G) in 2009 in humans. [27].

The bacterial strains of animal origin were resistant to tetracycline (approximately 100%) and also resistant to the third generation cephalosporin (6.82%) in 2009 in poultry.

Therefore, the *Salmonella* strains isolated from wastewater seems to resist to the third generation cephalosporin stronger than those observed in humans and animals, even if this fact is not statistically proved.

The bacteria strains, found in birds poultry were more resistant to tetracycline than were those observed in this study and in literatures [27]. This might be due to the low number of samples.

The transmission of antibiotic-resisting *Salmonella* strains from humans to animals and vice-versa is established; since they both live in the same environment that they contaminate. [31]

The presence of gene in the *Salmonella* spp, isolated from untreated wastewater, that gives him the capacity to resist to tetracycline was described by Zhang et al [32]. This, confirmed the role that could been played by the wastewaters in the transmission of the resistant genes between the *Salmonella* species and also between the human and animal population [32].

In Cote d'Ivoire, this relatively high percentage of *salmonella* sp. resisting to the third generation of cephalosporin, (C3G) found in humans, animals and in the environment, should be taken in account very carefully, since this antibiotic drug is commonly used in the treatment of infant salmonellosis [13]. In 2009, 8.1% of *salmonella* strains isolated from infants have resisted to the third generation of cephalosporin C3G [33]. World Health Organization, (WHO) reported the alarming increasing of the incidence of antibiotic-resisting *Salmonella*. This might be due wether to the abuse of antibiotic drugs in both human medicine and veterinary medicine [16] or to the presence of resistant *salmonella* strains in wastewater [34]. The genes of resistance to antibiotics are transferable between strains in the environment. That is another worrisome observation [32] [35]. Courvalin et al. believed that the antibiotic-resisting bacterias are the result of the modification of the genetic material (DNA). This gives them the capacity to grow in the presence of a antibiotic concentration superior to the quantity that normally inhibits them[36].

From what has been said, It is apparent that there is a progressive increasing of antibiotic-resisting *salmonella* strains from environment, humans and animals. [37]. Domestic and industrial effluents sometimes contain antibiotic residus [38]. This contributes strongly to the keeping, the evolution and also the spreading of the populations of antibiotic-resisting bacteria, initially exhibiting low level resistance [39].

Although, antibiotic-resisting strains were observed, the study showed a high level of sensitivity of all serovars to the eight tested antibiotic drugs across a broad spectrum ranging from phenicoles family (chloramphenicol), aminoglycosides (amikacin, gentamicin and kanamycin), quinolones (nalidixic acid and ciprofloxaxine) and a β -lactam particularly of the

278 Penicillins group has (ampicillin and amoxicillin & clavulanic acid). In case of salmonellosis,
279 any of the above-named drugs can be administered.

280 This limits of this study are due to the weak quantity of samples used (forty samples) and
281 also to weak quantity of strains (eleven strains). Therefore, it can not be generalized.

282

283 5. CONCLUSION

284

285 This study revealed, the presence of various *Salmonella* serotypes in wastewater
286 *Salmonella* Derby, *S. Essen*, *S. Farmsen*, *S. Hato* and *S. Ovonmouth*. It also showed out the
287 degree of resistance of these strains to commonly used antibiotic drugs. It also revealed that
288 the strains are resistant to 3rd generation cephalosporins (β -lactam antibiotics (cefotaxime)),
289 cyclin including tetracycline and antimetabolites (sulfonamide). Even though, 11.58% of
290 *Salmonella* strains resisted to antibiotics, *Salmonella* serovars remain totally sensitive to
291 phenicoles, aminoglycosides, quinolones and other β -lactam particularly the penicillins
292 Group A. These phenotypic characters of *Salmonella* allow to understand the challenges
293 related to the treatment of salmonellosis and also to understand the necessity on a rational
294 use of antibiotics.

295

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302

303

304 COMPETING INTERESTS

305

306 The authors declare that they have no competing interests

307

308 AUTHORS' CONTRIBUTIONS

309

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

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

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

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

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

318

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