

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

COULIBALY Kalpy Julien^{1*}, GADJI Alahou André Gabazé^{1,3}, KOUADIO Kouamé¹, KOFFI Kouadio Stephane² YAPO Ossey Bernard³, DOSSO Mireille¹

¹Institut Pasteur de Côte d'Ivoire ; Laboratoire de l'Unité d'Etudes et de Recherche de Contaminants Chimiques et Microbiologiques dans les Aliments (UNERCO)

²Laboratoire d'Environnement et de Biologie Aquatique ; Université d'Abobo-Adjamé

³Laboratoire des Sciences de l'Environnement; Université d'Abobo-Adjamé

ABSTRACT

Background: Recent studies have shown that wastewater is contaminated by salmonella sp., pathogenic bacteria resistant to antibiotics. The practice of the use of wastewater for irrigation purposes in the city of Abidjan is the likely source of food-borne diseases such as salmonellosis. However, knowledge of this resistant salmonella spp. in wastewater is limited within 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 university of Cocody main campus (Abidjan).

Methodology: Sampling was on a weekly basis. Wastewater samples were collected from four different sewers within the campus environment. Salmonella sp was isolated by standard laboratory method. 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 according to 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 for 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 high potency with 100% proven efficacy in the elimination of the salmonella sp.. Although, majority of the strains tested (85.94%) were eliminated by the antibiotics, the serotypes Derby, Hato and Farmsen showed resistance.

Conclusion: Wastewater in the main campus area of the University of Cocody contains the antibiotic-resistant strains of salmonella sp. In spite of the proven efficacy of some antibiotics in the elimination of salmonella strains, the resistance of these strains to third generation of cephalosporin and sulfamid is worrisome. It is recommended that further studies be carried out to determine the effects of these antibiotic-resistant salmonella species on human health.

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

1. INTRODUCTION

Developing countries are presently faced with serious problems of wastewater management owing to demographic explosion and poor urban planning. This lack of proper waste management system has negative influences on the environment and public health [1]. Abidjan, the Ivorian economic capital and the most populated city amongst the French-speaking West African countries is not spared from this ordeal. Wastewater which serves as a resource for these cities also poses a great threat [2]. Directly discharge into the sewerage network and in nature [3], without any prior treatment [4] [5], wastewater is used to irrigate crops, despite health risks [6], by the less privileged for which this practice is undeniably a source of revenue [7]. The composition of contaminants in wastewater is dependent on households' drinking water source and domestic water-use; these uses can bring a quasi-infinite number of pollutants especially the organic matter (urine, faeces) into sewers [8]. Water re-use in agriculture is the likely 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 infected individuals or healthy carriers are also constituents of wastewater in the sewage systems [2]. In Abidjan, it is 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 use of water contaminated with human or animal feces [8][10] [13]. Berge et al, reported that the abundance of salmonella in sewages if untreated before re-use poses 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 entero-bacteria [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].

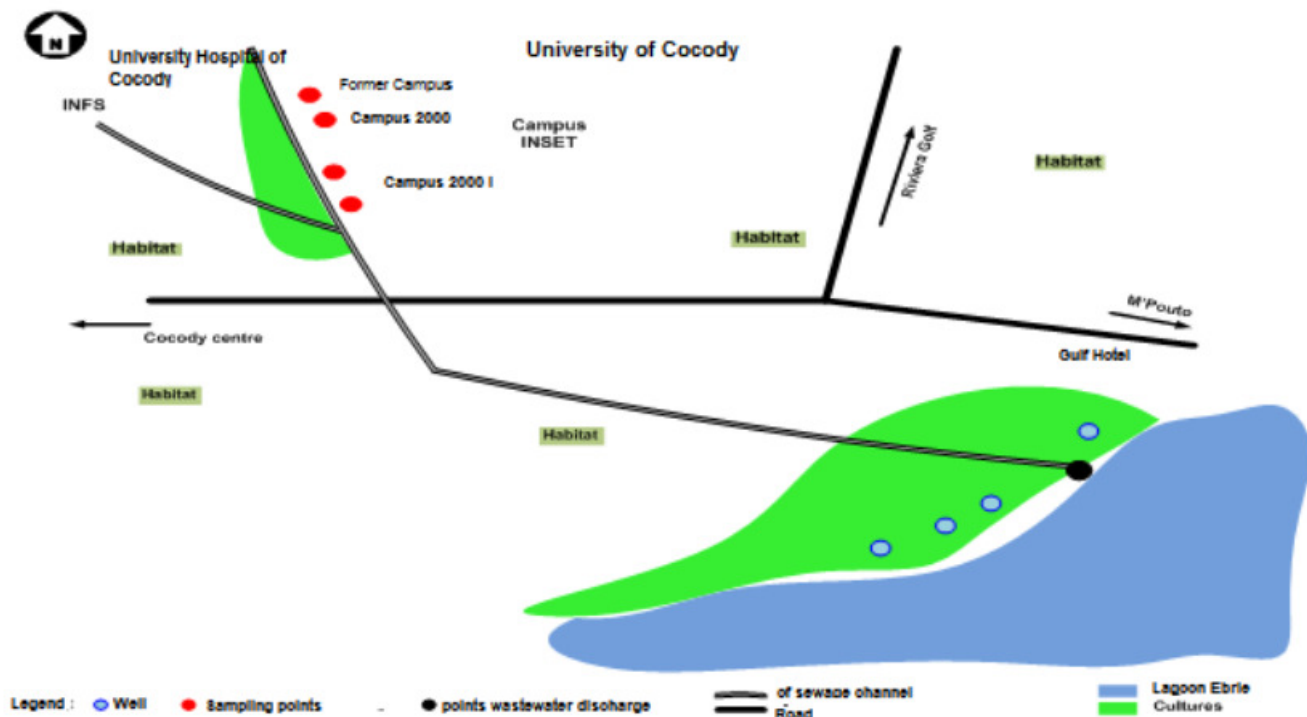
The main objective of this study is to establish the antimicrobial susceptibility profile of *Salmonella* spp. isolated from wastewater sewage of the Cocody University campus in Abidjan (Côte d'Ivoire) to antimicrobials commonly used in medicine to antimicrobials commonly used in human medicine.

2. MATERIAL AND METHODS

2.1. Study location

The campus of the University of Cocody is located in the commune of Cocody. It is home to an estimated 8,000 inhabitants and unaccountable thousands, daily accessing the area. The campus is surrounded by neighboring precarious settlements such as WASSA, CHU

69 village and CHU Baghdad. Households' wastes and wastewater from is directly discharged
 70 into the Ebrié lagoon (Figure 1).
 71 Sampling was conducted from August 2008 to January 2009.



72
 73 Figure 1: Map depicting the Cocody's communal area, the discharge channel of wastewater
 74 into the lagoon and the sampling sites of the study (Source: André GADJI, 2009)

76 2.2. Sampling methodology

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

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

84 2.3. Detection of Salmonella in sewage wastewater

85 The detection of Salmonella spp. was according to the conventional method of Edel and
 86 Kampelmacher [17] and retain by the international standards [18]. This involves 4 steps:

87 non-selective pre-enrichment, enrichment in selective media, the solid selective medium
88 isolation and biochemical and serological identification.

89

90 **2.3.1 Pre-enrichment in liquid medium**

91 10 mL sample was diluted by 90 ml of buffered peptone solution. The resulting mixture is left
92 to stand for about 3 min. A control (containing only peptone solution) was also run
93 simultaneously. They were subsequently incubated in an oven at 37 ° C for at least 16
94 hours in sterile plastics.

95

96 **2.3.2 Enrichment in selective liquid media**

97 0.1mL subsample is then collected and poured onto sterile tubes of 10mL of Rappaport
98 Vassiliadis media (RV10) and incubated at 42-44 ° C for 20 hours.

99

100 **2.3.3 Culture on selective isolation media**

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

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

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

111

112 **2.4. Evaluation of the antibioresistance [20].**

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

117

118 **2.4.1 Inoculum preparation**

119 Using Pasteur pipette one to two colonies were taken from a 24 hours' culture on regular
120 agar and transferred in a 2 mL saline suspension (0.9% NaCl) in a bottle. The solution was
121 homogenized with a VORTEX. Solution density was measured with a DENSIMAT to read

122 the turbidity. An amount of 100 μL of the solution is diluted into 10 mL of saline water to
123 adjust to McFarland 0.5 standard ($\sim 10^8$ cfu/mL).

124

125 **2.4.2 Seeding**

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

131

132 **2.4.3 Reading**

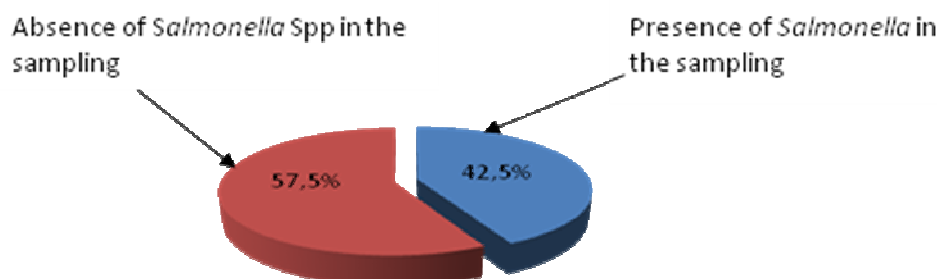
133 The Diameter of inhibition zones after 24 hours incubation at 37 °C is measured using a
134 foot slide for each disk and the values interpreted as (sensitive (S), resistant (R) or
135 intermediate (I) according to the CA-SFM instructions.

136

137 **3. RESULTS**

138 **3.1. The distribution of *Salmonella* sp in the water samples**

139 42.5% of the analyzed samples contained the salmonella sp. 36.36% salmonella Hato,
140 27.27% Farmsen, 18.18% Derby and 9.09% each of Essen and Ovonmouth, in order of
141 decreasing abundance. (Fig.3) This show the poor quality of these domestic effluents
142 directly discharged into the lagoon environments without any form of
143 treatment.



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

145

146

147

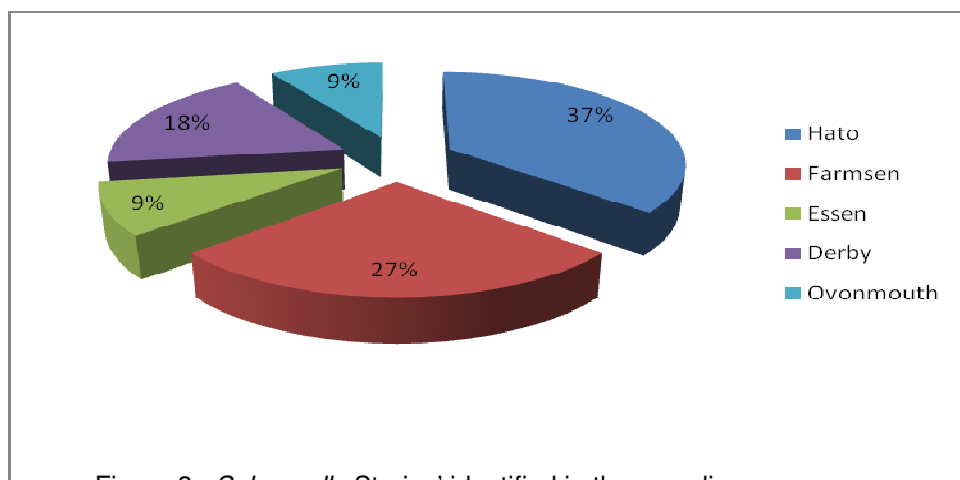


Figure 3 : *Salmonella* Strains' identified in the sampling

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).

163

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

170 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

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

172

173 3.3. The effectiveness of antibiotics and antimetabolite in the elimination of 174 the salmonella strains

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

183

184 4. DISCUSSIONS

185 The high abundance of Salmonella (42.5%) in wastewater from domestic sources implies a
186 high exposure risk and environmental pollution. Their abundance in the campus locality is
187 attributable to high population density and prevalent unhygienic and poor sanitary conditions.

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

190 Salmonella sp is primarily of intestinal origin and usually found in human excreta. Its
191 occurrence in the environment poses great health risks. In Cote d'Ivoire, Dongo et al. [3]
192 reported on the uncontrolled discharge of domestic effluents on the streets and in open
193 sewers. The risk associated with this irrational behaviour stems from their use or reuse in
194 agriculture in peri-urban area [22] and possible infiltration into subsurface waters under
195 hydrogeological condition [11].

196 The already identified 2400 Salmonella serovars worldwide are considered pathogenic.
197 Studies in Abidjan have shown occurrence of Salmonella sp in vegetables [23] and in water
198 used for irrigation purposes [24]. Also fresh vegetables collected from three different markets
199 within the commune of Port-Bouet, Koumassi and Cocody also showed the presence of
200 Salmonella. This is worrisome as according to Lorougnon [25], 44% of households with in
201 the city does not disinfect vegetables before consumption.

202 A vast majority of Salmonella present in the environment (soil, water, plant) comes from
203 animal and human excreta [26]. His therefore imperative that domestic effluents be treated
204 prior to their discharge in the environment. The uncontrolled disposal of effluents is
205 responsible for prevalent of diarrhea (19%) within the precarious settlements in Abidjan [3].
206 This allow favors the growth of Salmonella.

207 Salmonella sp. is at the origin of approximately 48% of food-borne diseases in Côte d'Ivoire
208 [27] and also in animals. It is an established fact that positive correlation exists between
209 salmonella sp. found in humans and animals, therefore, the presence of Salmonella strains
210 responsible for human infection in wastewater is worrisome. Indeed, the serotypes, Essen
211 and Farsem identified in this study have already been cited in human pathology in Côte
212 d'Ivoire and the Essen and Derby serotype in veterinary medicine. It is therefore conclusive
213 that in Côte d'Ivoire, that the Essen serotype can be found in humans, animals and
214 wastewater, other serotypes inclusive.

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

217 This study shows that the tested serovars are sensitive to all antibiotic drugs in common use
218 in human medicine. These include cyclin, phenicoles, quinolone, aminoglycosides and β -
219 lactam; except those of the 3rd generation. In fact, the study of Pilet et al. [14] showed that
220 Salmonella spp. is sensitive to most broad-spectrum antibiotics. However, resistance was
221 observed to cyclin notably tetracycline and the 3rd generation cephalosporin, whose
222 resistance pattern ranged between 9.1% and 18.2% respectively.. In general, there was
223 about 14.16% of salmonella bacteria showed resistance to all tested antibiotic drugs;
224 11.58% showed strong resistance while, 2.48% showed mild resistance.. This level of
225 resistance is similar to the findings of Al-Bahry et al. in Oman[28] but, lower than that of
226 Melloul [21] in Morocco; who isolated 36.5% resistant strains of salmonella in wastewater
227 All tested salmonella strains were resistant to sulfonamide (100%). Previous studies [29] and
228 those conducted by Bonny in Côte d'Ivoire [30] have highlighted the resistance of non-
229 typhoid salmonella strains to sulfonamide and tetracycline. , The percentage resistance to

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

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

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

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

242 The bacteria strains, found in poultry birds that are resistant to tetracycline are higher than
243 what was observed in this study and in literatures [27]. This might be due to the low number
244 of samples.

245 The transmission of Salmonella strains resistant to antibiotic from humans to animals and
246 vice-versa is no longer a topic of debate; as they both live in the same environment which
247 they contaminate. [31]

248 The presence of tetracycline resistance gene in Salmonella spp isolated from untreated
249 wastewater was described by Zhang et al [32]. Thus, affirming the role that could been
250 played by the wastewaters in the transmission of the resistant genes between the
251 Salmonella species and between the human and animal population [32].

252 In Cote d'Ivoire, this relatively high percentage of salmonella sp. resistant to the third
253 generation of cephalosporin, (C3G) found in humans, animals and in the environment,
254 should be given due attention, more so, as this antibiotic drug is frequently used in the
255 treatment of salmonellosis in infants [13]. In 2009, 8.1% of salmonella strains isolated from
256 infants have been resistant to the third generation of cephalosporin C3G [33]. World Health
257 Organization, (WHO) reported the alarming increase in the incidence of antibiotic-resistant
258 Salmonell. This might be due to the abuse of antibiotic drugs in human medicine and in
259 veterinary [16] or to the presence of resistant salmonella strain in wastewater [34]. The
260 genes of antibiotic-resistant strains are transferable between species in the environment;
261 another worrying observation [32] [35]. Courvalin et al. believed that the antibiotic-resistant
262 bacteria forms as a result of the modification of the genetic material (DNA). This allows
263 them to grow in the presence of antibiotic concentration higher than that which normally
264 inhibits them[36].

265 It is apparent from the foregoing that there is a progressive increasing of antibiotic-resistant
266 salmonella strains of environment, humans and animals. [37]. Domestic and industrial
267 effluents sometimes contains antibiotic residues [38]. This contributes strongly to the
268 maintenance, evolution and also spread of antibiotic-resistant bacteria populations, initially
269 exhibiting low level of resistance[39].

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

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

276 This study limitations are due to the small number of samples retained (forty samples) and
277 also the few number of isolated bacteria strains (eleven strains). Therefore, it cannot be
278 generalized.

279

280 5. CONCLUSION

281

282 This study highlights, the presence of various Salmonella serotypes in wastewater
283 Salmonella Derby, S. Essen, S. Farmsen, S. Hato and S. Ovonmouth. It also highlights the
284 levels of resistance of these strains to commonly used antibiotic drugs. It was revealed that
285 the strains are resistant to 3rd generation cephalosporins β -lactam antibiotics (cefotaxime),
286 cyclin including tetracycline and antimetabolites (sulfonamide). Despite showing a 11.58%
287 overall resistance to antibiotics, Salmonella serovars remain totally sensitive to phenicoles,
288 aminoglycosides, quinolones and other β -lactam particularly the penicillins Group A. The
289 phenotypic characteristics of Salmonella underscores the challenges related to the treatment
290 of salmonellosis and the rational use of antibiotics.

291

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298

299

300 COMPETING INTERESTS

301

302 The authors declare that they have no competing interests

303

304 AUTHORS' CONTRIBUTIONS

305

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

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

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

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

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

314

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