

SHORT RESEARCH ARTICLE

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

ABSTRACT

Aims: Study on the antimicrobial resistance pattern of the strains of *Salmonella* **spp** isolated from wastewater

Study design: spatio temporal

Place and Duration of Study: University campus of Cocody, Abidjan, Côte d'Ivoire, between August 2008 and January 2009.

Methodology: Seventeen strains of *Salmonella* were isolated by standard procedure from forty samples of wastewater. Antibiotics susceptibility testing was done on the strains of *Salmonella*.

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 sulfamid (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: The study has highlighted the presence of *Salmonella* in wastewater, varied serotypes, in this case *Salmonella* Derby, *S. Essen*, *S. Farmsen*, *S. Hato*, *S. Ovonmouth*, and their levels of resistance of strains towards usually used antibiotics

With the level of resistance which is 14.16%, the serotypes of *Salmonella* remain totally sensitive to **phenicoles**, to **aminosides**, to **quinolones** and in **β-lactamines**.

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

INTRODUCTION

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37 Big cities in developing countries (DCs) have a serious problem with the management of
38 wastewater discharges and their influence, direct or indirect, long or short term, on the
39 environment and public health (Ahoussi et al., 2008). Abidjan, the economic capital of Côte
40 d'Ivoire, does not escape this order (or situation).

41 Released into the environment without any treatment, the wastewater is used in irrigation of
42 market gardening, despite the sanitary risks, (Cissé, 1997) by disadvantaged populations for
43 which this practice generates significant financial resources (Camara, 1997). The final
44 composition of these waters is highly variable and contains many pathogenic microorganisms
45 including *Salmonella*. *Salmonella* are responsible for salmonellosis which is an infectious
46 disease of humans and animals, and the most frequent and important zoonotic disease
47 (Anonymous 1, 2005).

48 Studies have shown that the wild strain of *Salmonella* is characterized by sensitivity to all
49 antibiotics active against Enterobacteriaceae (Pilet et al., 1981; Anonymous 2, 2002).

50 However, there is to fear a progressive resistance of clinical strains or even environmental, in
51 view of the misuse of antibiotics in human medicine and animal husbandry (WHO, 2005).

52 The objective of our study was to establish the profile of antimicrobial resistance in
53 *Salmonella* isolated from sewage. The study was conducted at the campus of Cocody in
54 Abidjan, Côte d'Ivoire, at the channels of wastewater evacuation of the said campus. The
55 latter lead directly to a channel open, of the municipal sewerage system, which routes them in
56 the Ebrié lagoon.

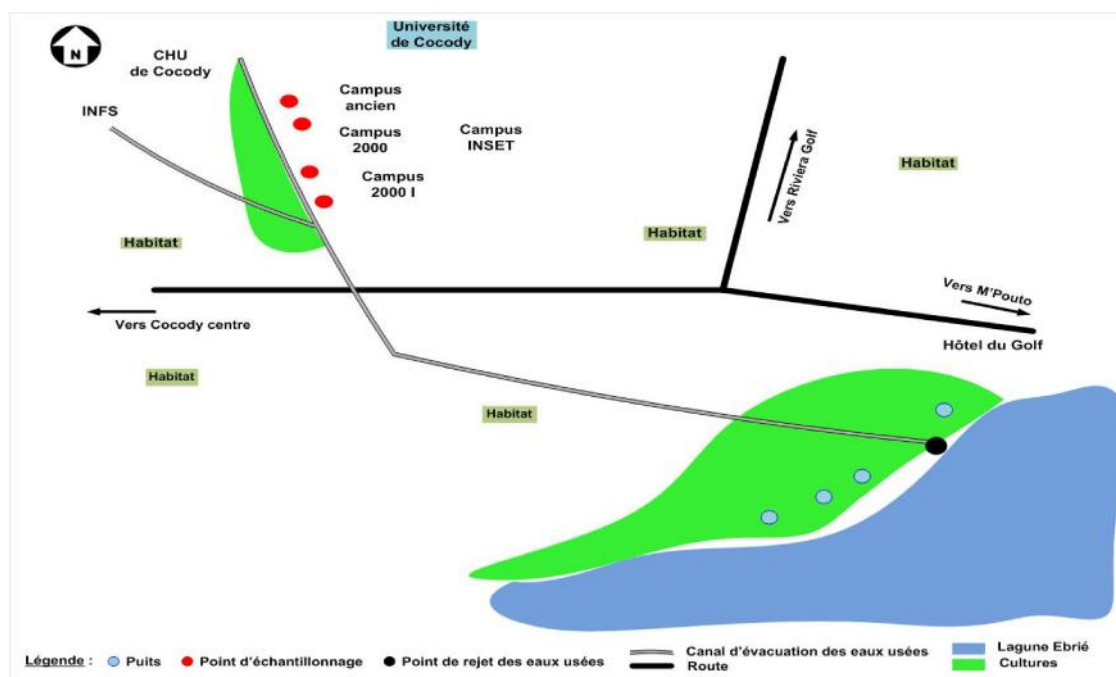
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58 MATERIALS AND METHODS

59 Setting of survey (or place of the study)

60 Located in the municipality of Cocody, the campus, which shelters at least 8,000
61 inhabitants not to mention the thousands of people who come in it, the campus includes OLD
62 campus, the INSET campus, the campus 2000 and the campus 2000-I. the Wastewater (toilet,
63 washing, cleaning, households, etc.) generated by the inhabitants are rejected by sewers
64 leading to a municipal manifold that routes them in the Ebrié lagoon, to the side of the Hotel
65 du Golf (fig. 1). This work is a study spatiotemporal began in August 2008 and ended in
66 January 2009.

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Picture 1: Localization of the points of withdrawal and the channel of evacuation of wastewaters in Lagoon
(source: André GADJI, 2009)

Sampling

Four (4) sewers were selected for sampling according to the criteria below:

Sewer capacity to drain a significant amount of wastewater

Their accessibility,

And their physical state (sewer in good condition).

The samples were collected using a sterile container and packed in sterile polystyrene bottles of 0.5 L. After collection, the samples were labeled (date and time of sampling, location, lot number), transported to the laboratory in a cooler with an ice pack and analyzed immediately. A total of 40 samples were collected in four (4) different sewers. The samples were taken every morning between 7 and 8 am, once a week over ten weeks. Each outing of sampling consisted of 4 samples (2 L of wastewater) including 1 sample by sewer.

Isolation and Identification of *Salmonella* spp

The method defines by Edel and Kampelmacher (1969) and adopted by the national and international standards (Humbert, 1998) was used in the bacteriological phase of this study.

All confirmed strains of *Salmonella* were subjected to the slide agglutination tests using agglutinating sera according to the Kauffmann-White diagram and completed by Le Minor (Bourgeois et al., 1996). After serotyping, sensitivity of strains to antibiotics was studied.

Evaluation of antibiotic resistance

89 The disk diffusion method in agar by Kirby Baur helped to determine the resistance
 90 or sensitivity of *Salmonella* strains. Eleven (11) antimicrobial chosen from five (5) different
 91 families were tested (Anonymous 2, 1996, Anonymous 3, 1993). These are: ampicillin,
 92 amoxicillin + clavulanic acid, cefotaxime, gentamicin, kanamycin, amikacin, ciprofloxacin,
 93 nalidixic acid, chloramphenicol and sulfonamide which is an antimetabolite.

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95 RESULTS

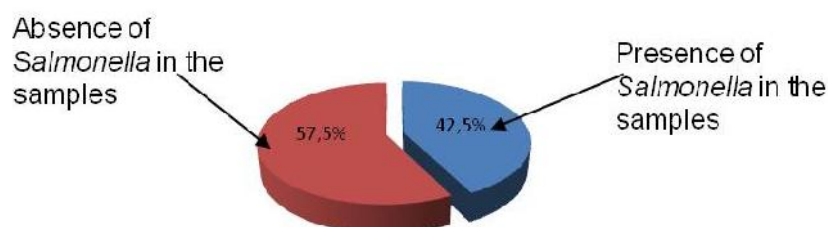
96 Identified *Salmonella* strains

97 Out of 40 analyzed samples, 17 revealed the presence of *Salmonella* and many strains were
 98 isolated (Fig. 2).

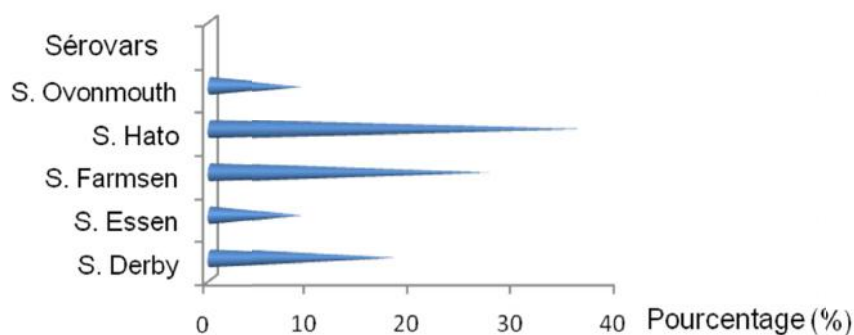
99 Five (5) serotypes were identified. It is about *Salmonella* Hato (4), *Salmonella* *Salmonella*
 100 Farmsen (3), *Salmonella* Derby (2), *Salmonella* Essen (1) and 1 *Salmonella* Ovonmouth (Fig.
 101 3).

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104 **Picture 2:** Proportion of the *Salmonella* in waters of sewers



105 **Picture 3:** Proportion of the strains of *Salmonella* identified

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107 Test of the antibiotic disks on the *Salmonella* serotypes

108 The test was performed with 10 antibiotics and 1 antimetabolite on 11 *Salmonella*
 109 serovars. The overall level of resistance is of 14.16% against 85.84% sensitivity. Specifically,
 110 the sulfonamide remained ineffective against strains with a level of resistance of 100%. As for
 111 cefotaxime, 5 serovars including *Salmonella* Ovonmouth, S. Hato, S. Derby and S. Farmsen
 112 resisted with respective resistance levels of 100% (n= 1), 50% (n = 2), 50% (n = 1), 33.33%
 113 (n = 1). The level of resistance for cefotaxime is therefore 46.67%. Concerning tetracycline,
 114 the level of resistance is 9.1%.

115 In contrast, the strains were sensitive to penicillin of group A, aminoglycosides, quinolones
 116 and phenicoles used. The table below shows the different levels of sensibility and resistance
 117 (Table I).

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120 Table I: Level of resistance and sensitivity of the *Salmonella* serotypes to the
121 antimicrobials

Antimicrobials	Number of strains	Number of sensitive	% Sensitive	% Resistant
AM	11	11	100	00
AMC	11	11	100	00
CTX	11	06	53.33	46.67
NA	11	11	100	00
GM	11	11	100	00
K	11	11	100	00
AN	11	11	100	00
CIP	11	11	100	00
C	11	11	100	00
TET	11	10	90.9	9.1
Antimétabolite				
SSS	11	00	00	100
Total			85.84	14.16

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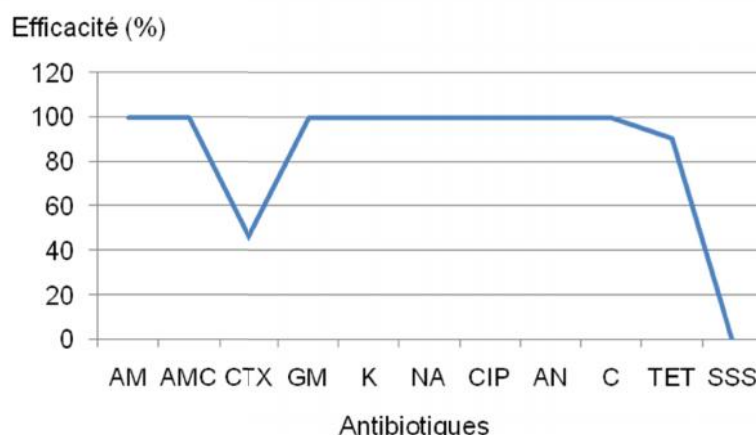
123 **Family of the β -lactamine:** ampicillin (AM), amoxicillin + clavulanic acidic (AMC) and cefotaxime (CTX); **family of the**
124 **aminosides:** amikacin (NA), gentamicin (GM) and kanamycin (K); **family of the quinolones:** nalidixic acidic (AN) and
125 ciprofloxacin (CIP); **family of the phénicolés:** chloramphénicol (C); **family of the cyclines:** tetracycline (TET); **families of**
126 **the sulfamides (antimétabolite):** sulfamide (SSS).
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Comparison of the efficacy of antibiotics

The performed test showed that the sulfonamide was the least effective, followed by cefotaxime and tetracycline with respective resistance levels of 100%, 46.67% and 9.1%. On the other hand, all strains were sensitive to ampicillin, amoxicillin + clavulanic acid, gentamicin, kanamycin, amikacin, ciprofloxacin, nalidixic acid and chloramphenicol. Figure 2 shows the evolution of the antibiotics tested according to the *Salmonella* strains isolated from sewage.



Picture 4: Curve of the efficiency of the antimicrobials

DISCUSSION

The abundance of *Salmonella* spp in domestic wastewater could give information about the risk to the health of the population and the environment of microbial pollution of these effluents. The presence of the pathogenic germ could be resulting to high bacterial pollution of wastewater and the local population would not be foreign to that (Sutra et al., 1998). Indeed, there is a high density of the population resides on the campus of Cocody and its surroundings, the level of sanitation and hygiene conditions are often precarious. According to Baumont (2005) the composition of domestic wastewater depends on three factors, which are the original composition of drinking water, the various uses by individuals who can make a near infinite number of pollutants, and finally the users themselves who are going to reject organic matter (urine, faeces) in the sewers. Although this bacterium is primarily of intestinal origin and usually found in sewage of human origin, its regularity in this environment poses a public health problem especially as these waters are discharged into the environment without treatment and often reused in Urban agriculture (Cissé 1997; Marchand and Martin, 1987). About this microorganism, the WHO has defined that the majority of 2400 *Salmonella* serovars should be considered potentially pathogenic. If it is proved that the wild strain is

characterized by sensitivity to all antibiotics active against Enterobacteriaceae (Anonymous 2, 2002), there is also to fear the emergence of resistant strains. Certainly our study has shown a very high sensitivity to 85.84% of the studied serovars tested against antibiotics, but resistance (14.16%) were observed vis-à-vis certain antibiotics. It is specifically sulfonamide, cefotaxime and tetracycline with respective resistance levels of 100%, 46.67% and 9.1%. In this regard, previous studies (Gatti et al., 1960; Bonny, 2006) have highlighted the resistance of non-typhoidal strains vis-à-vis the sulfonamide and tetracycline. Resistance levels found are respectively 11.59% and 93.75% for sulfonamides and 4.35% and 56.25% for tetracycline. This study has revealed higher level of resistances for sulfonamides while those of tetracycline were near the minima observed in the literature. The case of cefotaxime, a cephalosporin of the third generation (C3G), constitutes an alert of certainly misuse of this molecule. A resistance of *Salmonella* to C3G could compromise the use of Amino and carboxypénicilline in clinic even if they seem sensitive in vitro (EJ Threlfall et al., 2000). Indeed, the WHO (2005) noted that the alarming increase in the incidence of *Salmonella* strains resistant to antibiotics is due to the misuse of antibiotics in both human medicine and animal husbandry.

In recent years, it has been observed an increase in levels of resistance to certain serovars, such as *Salmonella* Typhimurium, *Salmonella* Hadar and *Salmonella* Saint-Paul, in relation to farming practices of livestock (Anonymous 2, 2002). Also, according to the same source, the use of new molecules of antibiotics in animal frequently leads to increased levels of resistance against these molecules.

In view of the above, there is reason to fear a progressive resistance environmental strains, but clinical resistance too (Baquero, 2001), given that the hydric environment contains antibiotic residues (Kouadio et al., 2009) which could also contribute significantly to the maintenance, the emergence and the spread of bacterial populations with a low level of resistance and ready to evolve towards the resistance (Berlau et al., 1999). However, while resistant strains were observed, the study showed a very high sensitivity level of all serovars to the eight antibiotics in a broad spectrum. It is about chloramphenicol, amikacin, gentamicin, kanamycin, nalidixic acid, ciprofloxacin, ampicillin and amoxicillin + clavulanic acid. They will certainly be the choice antibiotics in case of a salmonellosis.

CONCLUSION

This study has highlighted the presence of *Salmonella* in sewage, varied serotypes, namely *Salmonella* Derby, S. Essen, S. Farmsen, S. Hato and S. Ovonmouth and their level of resistance vis-à-vis the commonly used antibiotics in human medicine in Côte d'Ivoire. The study has revealed strains resistant to β -lactams particularly cephalosporin of 3rd generation (cefotaxime), to cyclins including tetracycline and sulfonamide including antimetabolites. However, with the level of resistance that is of 14.16%, the studied serotypes remain fully sensitive to phenicols, aminoglycosides, quinolones and to β -lactam especially to penicillin of group A. The phenotypic characteristics of *Salmonella* so found enable to define better the difficulties related to treatment of subjects (patients) with salmonellosis in the use of

antibiotics adapted to their affections. However, it remains imperious to work to maintain the level of sensitivity of antibiotics.

IN MEMORIUM

This article is dedicated to the doctor's MANIZAN memory

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