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Prevalence of *Salmonella* Strains in Cattle Breeding in the District of Abidjan (Côte d'Ivoire)

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A B S T R A C T

Salmonella is an enterobacterium present in the environment as well as in a large number of animals which, in most cases, serve as host asymptomatically. This bacterium is the leading cause of collective food poisoning in the world. In view of the expansion of salmonellosis in the world and the emergence of multi-resistant strains of *Salmonella*, this is a threat to human safety. The aim of this study is to determine the prevalence rate of *Salmonella* and their antibiotic resistance profile, antibiotics often used in cattle breeding in the district of Abidjan. In this study, we were able to analyze 420 samples of cattle fecal and 84 strains of *Salmonella* (20%) were isolated. Twenty-three (23) different serotypes were identified. Some of these strains were resistant to the antibiotics used. In view of the outbreak of salmonellosis and the emergence of multidrug-resistant strains *Salmonella*, biosecurity measures have to be adopted in cattle breeding in order to avoid the risk of food poisoning in the population.

Introduction

Salmonella is a gram-negative bacterium that belongs to the enterobacteria family (Yan *et al.*, 2003). It is present in the environment as well as in a large number of livestock which, in most cases, host it asymptomatically. This bacterium can survive in the environment for a very long period of time, thus contributing to the maintenance of the bacterium within the animal reservoir (Lailler *et al.*, 2015).

Salmonella is the main cause of collective Food Borne Disease Outbreak (FBDO) both in the developed and underdeveloped countries (Dubroca *et al.*, 2005; Bean *et al.*, 1997; Flint *et al.*, 2005; Karou *et al.*, 2013).

A high increase in infections caused by *Salmonella* present in human foods was noted in the United States, Europe and Korea. These

infections involved *Salmonella enteritidis* and *Salmonella typhimurium* (Brooks *et al.,* 2012; Janmohamed *et al.,* 2011; Jung *et al.,* 2007).

In most cases the food products, mostly contaminated are of animal origin, such as beef, poultry, milk or eggs, but any food, including fruits and vegetables can also be contaminated (Movassagh *et al.,* 2010).

In Côte d'Ivoire, beef as a source of animal protein occupies an important place in the daily diet of the population. In 2004, Ivorian total meat production was 171 000 tonnes and meat consumption requirements were covered at 94% by total meat production (Assoumy, 2009). This implied a higher risk of salmonellosis transmitted by cattle. In addition, salmonellosis in animals still presents a potential danger of zoonotic disease (Donough *et al.,* 1999).

In Côte d'Ivoire in general and in the district of Abidjan in particular, no data or information concerning the circulation of *Salmonella* in cattle breeding are available.

Faecal matter is a major source of contamination by direct contact or contamination of the environment. *Salmonella* contamination has enormous consequences, pathological, hygienic and economical consequences, due to the losses induced by animal and human pathologies on one hand and due to the high costs associated with *Salmonella* contamination control measures on the other hand (Coulibaly *et al.,* 2010).

In this context, *Salmonellae* constitute a serious threat to the health of our population, due to the spread of salmonellosis in the world and the emergence of multidrug resistant strains of *Salmonella.* The objective of this study was to determine the prevalence rate of *Salmonella* in cattle breeding in the district of Abidjan and their resistance profile.

Materials and Methods

Study area

This is a cross-sectional study on cattle breeding sites and livestock markets in five (5) municipals in the district of Abidjan: Port Bouët, Abobo, Adjamé, Yopougon and Bingerville. Two of these five municipalities are a popular cattle breeding sites (Port-Bouët and Bingerville), while the other three are mainly livestock markets (Abobo, Adjamé and Yopougon). In Port-Bouët, the samples were collected from three (3) sites, *i.e.,* at the farm, the slaughterhouse and from the livestock open market. These sites were chosen on the basis of their significant inflows of livestock destined for breeding, sale and consumption by the population of the district of Abidjan and also sincere cooperation of all the operators involved (farmers, sellers and owners of livestock) to participate in the study. The sites selected supplied a large amount of beef to the population of the district of Abidjan.

Sampling

The sampling consisted of collecting fresh cow dung from the cattle just after defecation on the various livestock sites. A total of 420 samples were collected from April to September 2016. During each exercise 30 cow dung samples were aseptically collected in sterile containers and transported to the microbiology laboratory of the Pasteur Institute of Cocody within one hour after collection, in an ice chest containing cold accumulator for analysis upon arrival.

Culture and identification of strains

All strains were isolated according to the protocol described by standard ISO6579 (2002E). Twenty-five (25) g of cow dung were pre-enriched in 225 mL of Buffered-

Peptone water (BPW) (Liofilchem®) in a stomacher bag and incubated at 37 °C for 24 hours. After 0.1 mL of pre-enriched broth was added to 10 mL of Rappaport Vassiliadis broth (RV 10) (Bio-Rad) and incubated at 44°C for 24 hours. From each Rappaport Vassiliadis suspension, Hektoen agar (OXOID®) in Petri dishes were streaked and incubated at 37°C for 24 hours. The suspected colonies retained exhibited morphological and biochemical characteristics as follows (Table 1).

The strains of *Salmonella* sp isolated were confirmed by MALDI-TOF Vitek MS (Bio Mérieux, France). *Escherichia coli* ATCC 8739 (Biomérieux, France) was used as a MALDI-TOF calibrating strain.

Serotyping of strains of *Salmonella* sp

Serotypes were determined by agglutination tests slide with anti-serum O, H and Vi (Bio Mérieux, France). Reading the results was made according to the Kauffmann White (1934) scheme.

Antimicrobial susceptibility testing

Salmonella strains were tested for their susceptibility to different antibiotics using Kirby-Bauer disc diffusion method (Bauer *et al.,* 1966). An inoculum was prepared with colonies of pure culture of 24 hours onto nutrient agar (Mueller Hinton agar) in Petri dishes. These colonies were emulsified in a tube of 10 mL of Salt water in order to obtain a homogenous suspension of density equivalent to 0.5 Mc Farland standards. A sterile swab was moistened in the bacterial suspension, and Miller-Hinton agar previously dried was seeded by swabbing the entire surface of agar by scoring tightened. The antibiotic disks were disposed onto the surface of the dried agar medium and the agar was incubated for 24 hours. After incubation, agar plats were read by measuring the diameters of inhibition zones around each antibiotic disk with the ADAGIO software (BioMérieux, France). The interpretation of the results was carried out according to the standard of the Antibiogram Committee of the French Society of Microbiology (CA-SFM / EUCAST 2014). A reference Strain of E. coli ATCC25922 (OXOID®) was tested as a quality a positive control for susceptibility testing. The following antibiotics were tested: Ampicillin (10 µg), Amoxicillin + clavulanic acid (30 µg), Cefalotin (30 µg), Cefepime (30 µg), Aztreonam (30 µg), Cefoxitin (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Imipenem (10 µg), Tetracycline (30 µg), Minocycline (30 µg), Gentamicin (15 µg), Tobramycin (10 µg), Amikacin 30 µg, Nalidixic acid(30 µg), Norfloxacin (5 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Colistin (50 µg) and Trimethoprim / Sulfamethoxazole (25 µg). The antibiotics were produced by Bio-Rad.

Results and Discussion

Prevalence of *Salmonella*

A total of 84 strains of *Salmonella* were isolated from the 420 cow dung samples tested, a prevalence of 20%. The municipality of Port-Bouët has the highest prevalence rate with 9.8%, followed by Adjamé (7%), Abobo (1.2%). The lowest prevalences were observed in Yopougon and Bingerville (Table 2).

Serotyping

The serotyping was carried out entirely for 33 strains. *Salmonella* isolates was distributed in twenty-three (23) different serotypes (Table 3).

S. muguga (2), *S. agbeni* (4), *S. II* (6), *S. virchow* (1), *S. IIIa* (2), *S. enteritidis* (1), *S. neumuenster* (1), *S. othmerschen* (1), *S.*

parkroyal (1), *S. senftenberg* (1), *S. umbadah* (1), *S. Dessau* (1), *S. tudu* (1), *S. muenster* (1), *S. gustavia* (1), *S. canton*, *S. salford* (1), *S. africana* (1), *S. bradford* (1), *S. chicago* (1), *S. erfut* (1), *S. bron* (1), *S. typhi* (1).

Antimicrobial susceptibility testing

Resistance to an antibiotic

A total of 54 strains of *Salmonella* was subjected to the antibiotic sensitivity test (antibiogram), 46.3% (25/54) of the strains exhibited resistance to at least one antibiotic. These strains showed resistance to colistin (24.1%), tetracycline (22.2%), minocycline (20.4%), trimethoprim / sulfamethoxazole (9.3%), nalidixic acid (7.4%), norfloxacin (5.6%), ciprofloxacin (5.6%), tobramycine (5.6%), cefalotin (3.7%), ceftazidime (1.9%), cefoxitin (1.9%), ceftriaxone (1.9%), amoxicillin + clavulanic acid (1.9%), aztreonam (1.9%), cefepime, Cefuroxim (1.9%) and Ampicillin (1.9%)

Phenotypes of resistant serovars

Of the 54 strains tested 22.22% of the strains (12/54) showed resistance to at least one antibiotic and 24.07% of the strains (13/54) showed resistance to at least 2 antibiotics (Tables 4 and 5). However, 53.7% of the strains (29/54) were sensitive to the antibiotics used (Table 3).

The prevalence of *Salmonella* in the cow dung samples tested in our study was 20%. This could be explained due to the fact that *Salmonellae* are naturally present in the digestive tract of warm-blooded animals (Bourgeois and Leveau, 1991).

In this study the prevalence (20%) is much higher than that reported by Fegan *et al*., (2004) in Australia, which was 6.88%. In the United States, the prevalence of *Salmonella* in cattle faeces in farms was reported to be less than 7% (Losinger *et al.*, 1997; Fedorka-Cray *et al.*, 1998; Dargatzet *et al.*, 2003). The presence of *Salmonella* in cattle could be linked, on one hand, to the cattle rearing method and, on the other hand, to the contamination of the breeding sites which are located in township therefore subjected to the influence of the activities of the population. This situation will encourage the circulation and spreading of *Salmonella*. In Côte d'Ivoire, no studies on *Salmonella* prevalence in cattle breeding have been reported, but studies by Traoré (2003) and Ouattara (2005) in poultry farming has reported *Salmonella* prevalence rates of 56% and 52 % respectively.

Table.1 Biochemical and morphological character of *Salmonella*

Biochemical and morphological character of *Salmonella*
$glc^{+}lac^{-}H_2S^{+}ur^{-}ind^{-}$ $LDC^{+}bg^{-}mob^{+}$ $gas^{+}cat^{+}$ ox^{-}

Glc^{+} = glucose fermentation; lac^{-} = no oxidation of lactose; H_2S^{+} = hydrogen sulfide production; ur^{-} = no production of urease; ind^{-} = no production of indole; LDC^{+} = production of lysine decarboxylase; bg^{-} = gram-negative bacilli; mob^{+} = presence of mobility; gas^{+}= gas production; cat^{+} = production of catalase; ox^{-}= no production of oxidase.

Table.2 Prevalence of *Salmonella*s isolated from the cow dung in the District of Abidjan

	Distribution of *Salmonella*s in the different municipalities					
	Abobo	Adjamé	Yopougon	Bingerville	Port-Bouët	Total
Number of sample	n= 60	n= 60	n= 60	n= 60	n= 180	420
Numberof *Salmonelle* **(n (%))**	**5(1,2%)**	**30(7%)**	**4(1 %)**	**4(1 %)**	**41(9,8%)**	**84(20%)**

Table.3 serotypes of isolated *Salmonella* spp strains

Serotype	Number
S. muguga	**2**
S. agbeni	**4**
S. II	**6**
S. IIIa	**2**
S.virchow	**1**
S. enteritidis	**1**
S. neumuenster	**1**
S. canton	**1**
S. othmerschen	**1**
S. parkroyal	**1**
S. senftenberg	**1**
S. umbadah	**1**
S. dessau	**1**
S. tudu	**1**
S. muenster	**1**
S. gustavia	**1**
S. salford	**1**
S. africana	**1**
S. bradfort	**1**
S.chicago	**1**
S.erfut	**1**
S.bron	**1**
S. typhi	**1**
Salmonella. sp	**51**

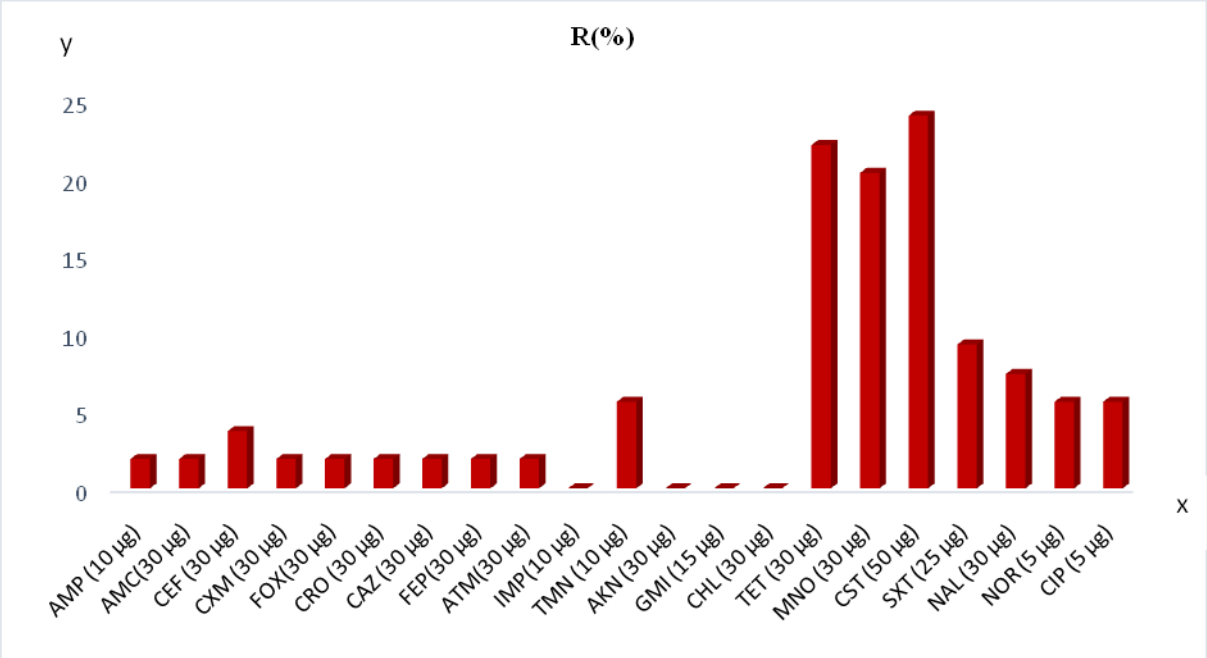
Table.4 Phenotypes of resistant strains to single antibiotic

Sample	Serotypes	Phenotypes
E44S	*S. enteritidis*	Tmn
E46S	*S. neumenster*	Cst
E67S/B	*S. senftenberg*	Sxt
E61S	*S. agbeni*	Cst
E74S	*S. dessau*	Sxt
E111S/B	*S.sp*	Cst
E45ADS/B	*S. sp*	Cst
E43ADS/C	*S. sp*	Cst
E43ADS/B	*S. sp*	Cst
E42ABS	*S. sp*	Cst
E73S	Umbadah	Tmn
E92S	*S. sp*	Cst

Table.5 Phenotypes of resistant strains to at least 2 antibiotics

Sample	Serotypes	Phenotypes
E64S	*S. II*	CefCstMnoTet
E32S	*S. virchow*	SxtMnoTetNalNorCip
E38S	*S. IIIa*	MnoTet
E60S	*S. sp*	CstTmn
E68S	*S. sp*	SxtMnoTet
E88S	*S. II*	NalNorCip
E89S	*S. gustavia*	TetMnoNalNorCip
E108S	*S. sp*	CstMnoTet
E28ADS	*S. sp*	CstMnoTet
E29PBS/B	*S. sp*	MnoNalTet
E66S	*S. sp*	MnoTet
E90S	*S. sp*	MnoTet
E28ABS/C	*S. sp*	CazFoxCroAmcAtmFepCepSxtCxmCstAmpMnoTet

Fig.1 Profile of resistance to antibiotics of *Salmonella* strains



AMP = Ampicillin, AMC = Amoxicillin + clavulanicacid, CEF = Cefalotin, FEP = Cefepime, ATM = Aztreonam, FOX = Cefoxitin, CRO = Ceftriaxone, CAZ = Ceftazidime, CXM = Cefuroxime, IMP = Imipenem, TET = Tetracycline, MNO = Minocycline, GMI = Gentamicin, TMN = Tobramycine, AKN = Amikacin, NAL = nalidixicacid, NOR = Norfloxacin, CIP = Ciprofloxacin,CHL = Chloramphenicol, CST = Colistin et SXT = Trimethoprim / sulfamethoxazole

Salmonella strains subjected to serotyping gave 23 different serotypes and also showed a predominance of *Salmonella II* (6/33). The frequency of isolation of serovars varies according to region and even country Khar (2006). Resistance profiles obtained in this

study showed a resistance rate of 22.2% for colistin, 16.7% for tetracycline, 16.7% for minocycline, 7.4% for trimethoprim / sulfamethoxazole, 7.4% for nalidixic acid, 5.6% for norfloxacin, 5.6% for ciprofloxacin, 3.7% for tobramycin and 1.9% for cefalotine (Fig. 1). Study results do not corroborate to that of Ahi *et al.*, (1990), who, according to him between 1980 and 1988, all *Salmonellae* isolated in Côte d'Ivoire were sensitive to fluoroquinolones, on the other hand, these results are in agreement with the works of Coulibaly *et al.*, (2009). *Salmonellae* are enterobacteriaceae (Yan *et al.*, 2003), naturally sensitive to betalactamins, aminoglycosides, quinolones, fluoroquinolones and nitrofurantoin. Therefore, strains sensitive to all families of antibiotics could be purely wild strains. This would explain why the acquisition of a multiresistance character would be caused by the misuse of antibiotics in the prophylactic and metaphylactic treatments of animals. Resistance to colistin was rarely observed despite selection pressure (Jensen *et al.*, 1987, Pit *et al.*, 2003), it remains the last choice antibiotic when the use of an antibiotic of the betalactamin family Aminoglycosides, or quinolones seems to be inefficient (Livermore, 2002). The resistance to colistin would come from the contamination of the herds of their immediate environment.

In conclusion, this study showed a high prevalence of *Salmonella* in cattle rearing. Contamination of the environment by these germs can cause the occurrence of infectious diseases in humans and in cattle. For this purpose, further study on *Salmonella* in cattle should be taken in order to determine their pathogenic factors. To check the transmission and spreading of *Salmonella* in cattle breeding biosecurity measures must be taken. Finally, the use of antibiotics in cattle breeding should only be done if necessary to avoid resistant strains of *Salmonella* to antibiotics.

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