Study Of The Antibiotic Resistance Profile Of Escherichia Coli And Salmonella Spp. Isolated From Cattle Dung At The Port-Bouët Slaughterhouse (Abidjan, Ivory Coast)

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Abstract: The overconsumption of antibiotics by livestock represents a productivity gain for the agri-food industry; but the emergence and growing development of bacterial antimicrobial resistance is a global public health problem that is hurting the agri-food industry. This work aims to evaluate the use of antibiotics in farms and to determine the antibiotic resistance profile of strains of E. coli and Salmonella spp. isolated cattle dung at the abattoir of Port-Bouët (Abidjan, Côte d'Ivoire). Studies have been conducted to identify and identify the desired bacteria. Then, the sensitivity of the isolated bacterial strains to the antibiotics was determined by the standard method of diffusion on Mueller-Hinton agar medium. Samples (77) of bovine dung were collected and bacteriological analysis identified 23 strains of E. coli and 4 strains of Salmonella spp. used for the antimicrobial resistance test. Of the 77 cattle dung samples collected, the results showed the presence of resistant E. coli in 23 or 29.86% isolates and 4 isolates, or 5.19% of Salmonella spp., Of which 23 were E. coli strains tested. at 16 of the 21 antibiotics used, a resistance level of 71.19%, the 4 isolates of Salmonella spp. were mostly resistant to 4 antibiotics ie 19.04%. The highest levels of E. coli resistance were observed with β-lactams (100%) with the exception of Cefoxitin, Imipenem and 100% Tetracycline. For Salmonella spp. these levels were observed with Tetracycline (100%) and Ciprofloxacin (50%). The resistance phenotypes and the resistance rates observed on the isolates obtained at the Port-Bouët abattoir encourage increased surveillance of the use of antibiotics in the cattle industry in Côte d'Ivoire. This study revealed the presence of Salmonella spp. and important strains of E. coli in bovine dung, but mostly revealed the resistance of the strains to different antibiotics with high rates of resistance. Resistance having a considerable impact on the health of consumers causing many pathologies (Urinary tract infections, Typhoid fever, Food toxi-infection).

Keywords: E. coli, Salmonella spp., Antibiotic, resistance, cattle

1. Introduction

The discovery of penicillin by Alexander Fleming in 1928, followed by the search for new antibiotics and their use in human and veterinary medicine, was a major scientific advance in the twentieth century. Many bacterial infectious diseases, pests for humans and livestock, were thus combated. The evolution of society and agricultural and industrial systems led, in the 1950s, to an increasingly important and sometimes excessive use of antibiotics in humans and animals[1].. The problems related to this use appeared very early: as early as the 1960s, bacterial resistance was described in the context of hospital epidemics (Klebsiella pneumoniae, Escherichia coli), as well as possible transitions of multidrug-resistant bacteria between the animal and Human (Salmonella enterica) [2]. . Therefore, discussions on the reasoned use of antibiotics in humans as in animals have been initiated **3**. But given their therapeutic value, the use of antibiotics continued its exponential curve at the world level until the end of the nineties. The first European recommendations on a reasoned use of antibiotics to fight against the appearance of bacterial resistance were issued in 1998 at the Copenhagen conference. Currently, in Europe (France), the first national antibiotic plan for the fight against antimicrobial resistance in human and veterinary medicine is planned for the period 2018-2022 [4]. This work provides the answers to the most frequently asked questions concerning the current uses of antibiotics. antibiotics in farm animals. In these animals antibiotics are administered therapeutically, preventatively and to accelerate their growth thus representing a productivity

gain for the agri-food industry. But the misuse or inappropriate use of these antibiotics makes farms a privileged place for resistant pathogens to appear, develop and spread. [5]. A study was therefore conducted to isolate and identify E. coli and Salmonella spp. Frequently occur in these farms to determine their antibiotic resistance profile and to assess the impact of this resistance on the health of cattle and the consumer. Contamination with bacterial strains from animals can cause serious pathologies in humans. This contamination occurs through the consumption of stale animal products. It can also be done by contact with the environment, contaminated by live animals via feces, manure, manure or via effluents from slaughterhouses. [6]. Slaughterhouse effluents correspond to all the liquid discharges produced on the site. slaughterhouses, ie water resulting from slaughtering (process, washing) and sewage (sanitary). By their nature, these effluents are heavily loaded with bacteria [7]. Studies indicate that bacteria resistant to antibiotics and / or pathogens of human or animal origin such as Escherichia coli, Listeria monocytogenes, Salmonella spp., Campylobacter spp. Are excreted into the environment via slaughterhouse water. [8]-[10].. Ruminants, the main reservoir, would participate in the maintenance of the epidemiological cycle of pathogens [11], [12]. The intensive use of antibiotics for therapeutic, preventive or growth-stimulating purposes raises livestock, particularly that of cattle in privileged places so that resistant pathogens can grow, survive and spread [13]. Indeed, Escherichia coli and Salmonella spp are the most frequently isolated bacteria in these farms. Studies in several countries show that these bacteria develop resistance to certain antibiotics. Faced with this situation, these countries have developed surveillance networks on the antibiotic resistance of these strains.Located in the south of Ivory Coast, the city of Abidjan is the first city of Côte d'Ivoire in terms of inhabitants with a population estimated at more than 6 million inhabitants. It has only one modern slaughterhouse, that of the municipality of Port-Bouët, which represents the largest slaughterhouse in the country on an area of 6 ha. Unfortunately, the Port-Bouët slaughterhouse is not equipped with a pre-treatment or wastewater treatment system. The effluents are dumped without treatment in the Ebrié lagoon which is used by the surrounding populations for fishing, market gardening ... Thus, the liquid discharges of the slaughterhouse would represent one of the main environmental problems to which the municipality of Port-Bouët in However, the city of Abidjan in general would be confronted, given the magnitude of the pollution generated by these effluents that could have an impact on the surface water resources and the health of the populations. Faced with this situation and in the context of the control and surveillance of diseases caused by resistant pathogens, it was considered interesting to carry out a study on the wastewater from the Port-Bouët slaughterhouse. The main objective of this study is to isolate strains of Escherichia coli and antibiotic-resistant Salmonella spp. From cattle dung from the Port-Bouët slaughterhouse. To this end, there are two specific objectives, which are initially to isolate the Escherichia coli and Salmonella spp strains from cattle dung of the Port-Bouët slaughterhouse, and secondly to determine the antibiotic resistance of these strains

2. Materials & methods

2.1 Samples

Sampling was done on slaughtered cattle. It consisted in taking dung from the rectum of these cattle. For this purpose, 77 dung of diarrheal cattle including 49 from bulls, 27 from cows and one calf were collected at the slaughterhouse of Port-Bouët from 03 to 24 December 2017. The samples of dung carried an information sheet distinguishing the coordinates following: sample number, place of collection, date and time of collection, sex of animals, age of animals, appearance of the collected dung (Solids, diarrhea) and level of sampling. The pots containing the various faeces were transported in a cooler containing cold accumulators to the Institut Pasteur laboratory in Côte d'Ivoire for bacteriological analyzes.

2.2. Cultivation and identification

Bovine dung samples were used in the identification and resistance pattern of E. coli and Salmonella spp. Growth media chosen are Rapid'E Coli 2 medium (REC2) supplemented with ceftazidime for E. coli strains and Hektoen medium for Salmonella spp. For the identification of strains; a bacterial suspension was prepared. For this purpose, one gram (1g) of cattle dung was weighed, then 9 ml of Buffered Peptone Water (EPT) was added to the tubes containing the sample. The whole was homogenized for two minutes thus constituting the bacterial suspension. The search for E. coli was carried out according to the method described by Baguy et al., 2014 14. Thus the bacterial suspension previously prepared was inoculated on the REC2 medium supplemented with 2 mg / L of ceftazidime and then incubated at 44 ° C. for 24 hours. The search for strains of Salmonella spp. was made according to the standard NF EN ISO 6579-1 (2017). It consisted in taking 0.1 ml of the bacterial suspension incubated at 37 ° C. in 24 hours to 10 ml of Rappaport Vassiliadis (RV 10) (Bio-Rad) incubated at 44 ° C. for 24 hours followed by isolation. by streak on Hektoen agar. Colonies of violet-colored E. coli and translucent Salmonella spp. With or without a black center are used for biochemical identification.

2.3. Sensitivity to antibiotics

Strains of E. coli and Salmonella spp. were tested for their sensitivity to 21 antibiotics by the method of diffusion of antibiotic discs in agar medium. An inoculum was prepared with 24-hour pure culture colonies on Nutrient Agar (Mueller Hinton Agar) in Petri dishes. These colonies were emulsified in a 10 ml tube of salt water to obtain a homogeneous suspension of density equivalent to 0.5 Mc Farland standard. A sterile buffer was wetted in the bacterial suspension and previously dried Miller-Hinton agar was seeded with a rotary seed. The antibiotic discs were placed on the surface of the dried medium and the agar was incubated for 24 hours at 37 ° C. After incubation, the agar plates were read by measuring the diameters of the inhibition zones around each antibiotic disc with the ADAGIO software (Bio Mérieux, France). The interpretation of the results was carried out according to the method described by the Antibiogram Committee of the French Society of Microbiology (CA-SFM / EUCAST 2017) 15. The following antibiotics were tested: Amoxicillin + clavulanic acid (30 µg), Ceftazidime (30

µg), Aztreonam (30 µg), Cefoxitine (30 µg), Cefixime (5 µg), Imipenem (10 µg), Ampicillin (30 µg), Ceftriaxone (10 µg), Cefepime (30 µg), Ciprofloxacin (5 µg)), Nalidixic acid (30 µg), Norfloxacin (10 µg), Gentamicin (10 µg), Amikacin (30 µg), Tobramycin (10 µg), Tetracycline (30 µg), Minocycline (30 µg), Colistin (50 µg) Cefotaxime (5 µg), Chloramphenicol (30 µg), Sulfamethoxazole / Trimethoprim (25 µg). Antibiotics were produced by Bio-Rad

3. Results

3.1. Isolation and identification

A total of 77 samples were analyzed at the Institut Pasteur in Côte d'Ivoire. Of these 77 samples, 23 E. coli strains of which 13 (26.53%) from bulls and 10 (37.04%) from cows accounted for 29.87% of all identified E. coli strains. . 4 salmonella strains were identified with only bulls with 5.19% of all identified samples and 50 samples without E. coli and Salmonella spp. a proportion of 64.94%.

3.2. Antibiotic sensitivity study

A total of 27 strains of E. coli and Salmonella spp. have been tested for their sensitivities to 21 molecules of antibiotics of different families.

3.2.1. ß-lactam antibiotics

The 23 strains of E. coli tested showed resistance to betalactamines. Of these, 17.39% (4/23) are resistant to second-generation cephalosporins (cefoxitin) and 100% (23/23) are resistant to third-generation penicillins and cephalosporins (ampicillin, amoxicillin + clavulanic acid). , cefixime, cefepime, aztreonam, ceftriaxone, ceftazidime, and cefotaxime). Imipenem remains the only antibiotic of this susceptible family on all strains tested (Figure 1). The 4 strains of Salmonella spp. tested showed resistance to betalactamines. Of these, 75% (3/4) are susceptible to third-generation cephalosporins such as ceftazidime and ceftriaxone. Imipenem, penicillins and other second- and third-generation cephalosporins (ampicillin, amoxicillin + clavulanic acid, cefoxitin, cefixime, cefepime, aztreonam) show 100% sensitivity for the four strains tested (Table II).

3.2.2. Aminoglycosides

Aminoglycosides have a very good efficacy on all E. coli strains tested in our study with 100% (23/23) sensitivity to gentamicin and amikacin (Table I). Tobramycin with three resistant strains and two intermediate strains had rates of 13.04% and 8.69%. Gentamicin, tobramycin and amikacin have a sensitivity of 100% (4/4) for all strains of Salmonella spp. (Table II)

3.2.3. Cyclins

With regard to cyclins, the most important resistances of E. coli strains are observed with tetracyclines which show 100% (23/23) resistant strains (Table I) Minocyclines show 56.52% (13/23) of susceptible strains and 13.04% (3/23) of intermediate strains. Salmonella has the highest level of resistance with tetracyclines 75% (3/4) and a rate of 25% (1/4) for minocycline (Figure 2)

3.2.4. Quinolones

The resistance of E. coli strains to quinolones is 43.48% (10/23) for nalidixic acid 47.83% (11/23) for norfloxacin and 39.13% (9 / 23) for ciprofloxacin (Figure 1). Regarding strains of Salmonella spp. quinolones have resistance rates of 25% (1/4) for nalidixic acid and 50% (2/4) for ciprofloxacin; for this family, Norfloxacin appears to be the appropriate treatment with a sensitivity rate of 100% (4/4) (Figure 2).

3.2.5. Others antibiotics

Phenicolates including Chloramphenicol has 100% (23/23) sensitivity to all strains of E. coli studied. The sulfonamides have 47.83% (11/23) of resistance (Table I). As for Salmonella, Chloramphenicol and Sulfamides have sensitivity rates of 100%. Colistine has 25% (1/4) of susceptible strains (Figure 2). ets [1]–[3]. Please note that the references at the end of this document are in the preferred referencing style. Please ensure that the provided references are complete with all the details and also cited inside the manuscript (example: page numbers, year of publication, publisher's name etc.).

4. Discussion

Escherichia coli and Salmonella spp are part of a heterogeneous family of Gram-negative bacteria that is frequently implicated in human infections. These germs are mostly pathogens of the human digestive tract and others are normal colonizers of this digestive tract. [16] In this study, 77 dung of diarrheal cattle including 49 from bulls, 27 from cows and one calf were analyzed, 23 strains of Escherichia coli including 10 from cows and 13 from bulls, respectively 37.04% and 26.53% respectively. The prevalence of E. coli on all samples analyzed was 29.87%. 4 strains of Salmonella spp were identified with a prevalence of 5.19% on all samples (77). The presence of Escherichia coli and Salmonella spp observed in the present work can be normal because these germs constitute 80% of the intestinal flora. The results of the bacteriological analyzes reveal a strong presence in the stool of Escherichia coli with higher prevalences in stools from cows. Strains of Salmonella spp were more prevalent in stools from bulls. These results are consistent with those obtained in Spain by [17] in cattle where the percentage isolation of Escherichia coli was 48%. The presence of Salmonella spp. Also highlighted in 5.19% of the samples is consistent with the work of [18] which obtained prevalences of 5% in the Port Bouët cattle parks. In addition, studies carried out by [19] on cattle in the city of Abidjan (Côte d'Ivoire) reveal a rate of 20% for Salmonella. All this reveals that the animals are reservoirs of Escherichia coli and Salmonella spp and that these bacteria live in the digestive tract of animals such as cattle. In livestock farming, non-compliance with good hygiene practices may be the main source of contamination of animals by bacteria. Some studies, particularly those of [13], showed that farmers did not comply with sanitary prophylaxis measures (poor cleaning, poor disinfection, lack of crawlspace), which allowed the bacteria to remain in the breeding [20]. These hygienic indicator microorganisms lead to carcass contamination involving defective hygienic practices, particularly in slaughterhouses. Indeed after bleeding, the animals pass in

tanks of scalding. The water in these tanks is very loaded with organic matter (dung, blood). The microorganisms can survive and as the carcasses pass the microbial load increases despite the renewal of water, which can lead to cross contamination. The passage of several animals from different farms can, through these same vectors, accentuate this microbiological risk [21]. Plucking, evisceration, washing, are stages of preparation that participate in the entire process of contamination. The antibiotic sensitivity profile of the 23 strains of E. coli studied shows a very high resistance (100%) to most β-lactams (penicillin, third-generation cephalosporin) except imipenem which remains sensitive on all strains studied, indicating its place of choice in the treatment of severe infections with multi-resistant bacteria. The resistance of E. coli revealed to third generation cephalosporins (cefotaxime, ceftazidime, cefepime, cefixime) in this study was present at 100% prevalence. This is to say that the use of antibiotics, including third-generation cephalosporins for therapeutic purposes, is the most important risk factor in the development of bacterial resistance, [22] which has become a major public health problem. This resistance to cephalosporins has also been demonstrated in Belgium in poultry [23]. Some authors have also shown that the isolation of cephalosporin-resistant strains of E. coli is present in different animal species. [24] Similar results have been found in an Algerian study, where the percentage of resistance of strains to β-lactams was also 100% to the majority of third-generation cephalosporin antibiotics tested [25]. The β-lactam resistance observed in the present study implies that E. coli strains produce beta-lactamases which inactivate these antibiotics by hydrolysis of the beta-lactam nucleus, these results being in agreement with those of [26], which showed this resistance to β-lactams. Also, note that significant resistance is observed with aminosides (tobramycin 13.04%), fluoroquinolones (43.48% for nalidixic acid, 47.83% norfloxacin and 39.13% for ciprofloxacin), cyclins (100% resistance for tetracyclines) and sulfonamides (47.83%). This is related to the misuse of broad-spectrum antibiotics (penicillins, cephalosporins, chloramphenicol, tetracyclines, fluoroquinolones, aminoglycosides, sulfonamides). These results are similar to those of [27] who isolated E. coli strains from Port-Bouët livestock pens. Similarly, the proportions found by [28] in a study of enterobacteriaceae producing extended-spectrum beta-lactamases (ESBL) isolated at the Tunisian university hospital, are also close to those observed with 100% resistance to third-generation cephalosporins. and 67.5% to fluoroquinolones [29]. The evaluation of the sensitivity of salmonella to antibiotics revealed resistance to β-lactam antibiotics (ceftriaxone 25%, ceftazidime 25%), fluoroquinolones (nalidixic acid 25%, ciprofloxacin 50%), colistin 25% and cyclins (75 % tetracyclines, 25% minocyclines) with varying levels and profiles, accompanied by expression of a plasmid cephalosporinase phenotype with ceftazidime. This situation reflects a risky use of these molecules as a growth promoter in the meat sector [29] and in veterinary therapy, a situation that prompted WHO in 2001, organized a new consultation on the impact of the use of antibiotics, mainly quinolones, in food animals in human medicine following the increase of Salmonella strains resistant to nalidixic acid [30]. The strains of Salmonella studied showed sensitivity to several antibiotics tested proving that wild strains of Salmonella are naturally sensitive to all the antibiotics active on enterobacteria [31] which could explain this sensitivity observed in our study on several antibiotics. The results obtained are less alarming than those observed in other countries of Europe where the evolution of resistance has affected several antibiotics. In France, for example, data from 2008-2009 on the active surveillance of salmonella resistance to antibiotics in the meat sector showed that among the strains on which a sensitivity study has been carried out, certain strains have multi-resistant phenotypes of chloramphenicol, streptomycin, ampicillin, tetracyclines and sulfonamides [32]. In this study, strains of Salmonella spp. showed resistance to at least one antibiotic with levels lower than 13; However, the evolution towards resistance is growing, which is alarming in a country like Côte d'Ivoire where animals (cattle) play an important role in the daily diet of the population. This situation makes it difficult to choose effective measures to limit the spectrum of action of antibiotics, because it is necessary to avoid the improper use of antibiotics without being able to control the spread of resistance.

5. Conclusion

The objective of this study was to determine the antibiotic resistance profile of strains of E. coli and Salmonella spp isolated from cattle dung at the Port-Bouët slaughterhouse. During the 6 months of this study, 77 samples were analyzed and identified at the Institut Pasteur in Côte d'Ivoire. Of the 77 samples analyzed, 23 strains of E. coli and 4 strains of Salmonella spp. were isolated and identified with a respective prevalence of 29.87% and 5.19%. The determination of the antibiotic resistance profile showed a very high resistance (100%) of the 23 strains of E. coli to the beta-lactam family, with the exception of imipenem which remains active on all the strains tested. In addition, there are significant resistances with cyclins (100% tetracyclines), quinolones (47.83% norfloxacin), sulfonamides (47.83%). Strains of Salmonella showed high sensitivity (100%) on more than half of the antibiotics tested (14 antibiotics) with some resistance to the family of beta-lactams (25%), cyclins (75% tetracyclines), quinolones (50% ciprofloxacin). In sum, this study revealed the presence of Salmonella spp. and important E. coli strains in cattle dung at the Port Bouët abattoir, but above all it revealed the resistance of the strains to the different antibiotics with high resistance rates, which seems alarming for a country like Côte Ivory. It should, like developed countries, set up a surveillance network for the antibiotic resistance of bacteria of animal origin, a prerequisite for apprehending this complex and constantly evolving problem. Only such an approach will allow antibiotics to keep their valuable efficiency and to continue to render in the animal pathology of tomorrow the services they have rendered in the recent past. It would be interesting to select a more representative number of strains of E. coli and Salmonella spp. The repetition of this work should allow in the future to evaluate the impact of the spread of resistant bacteria on the health of cattle and those consumers. Nevertheless, these strains need to be quickly and efficiently detected in order to take the appropriate preventive and therapeutic measures as quickly as possible. Their detection can sometimes be

difficult, but new serotyping and molecular biology techniques are powerful and robust tools for their detection and identification.

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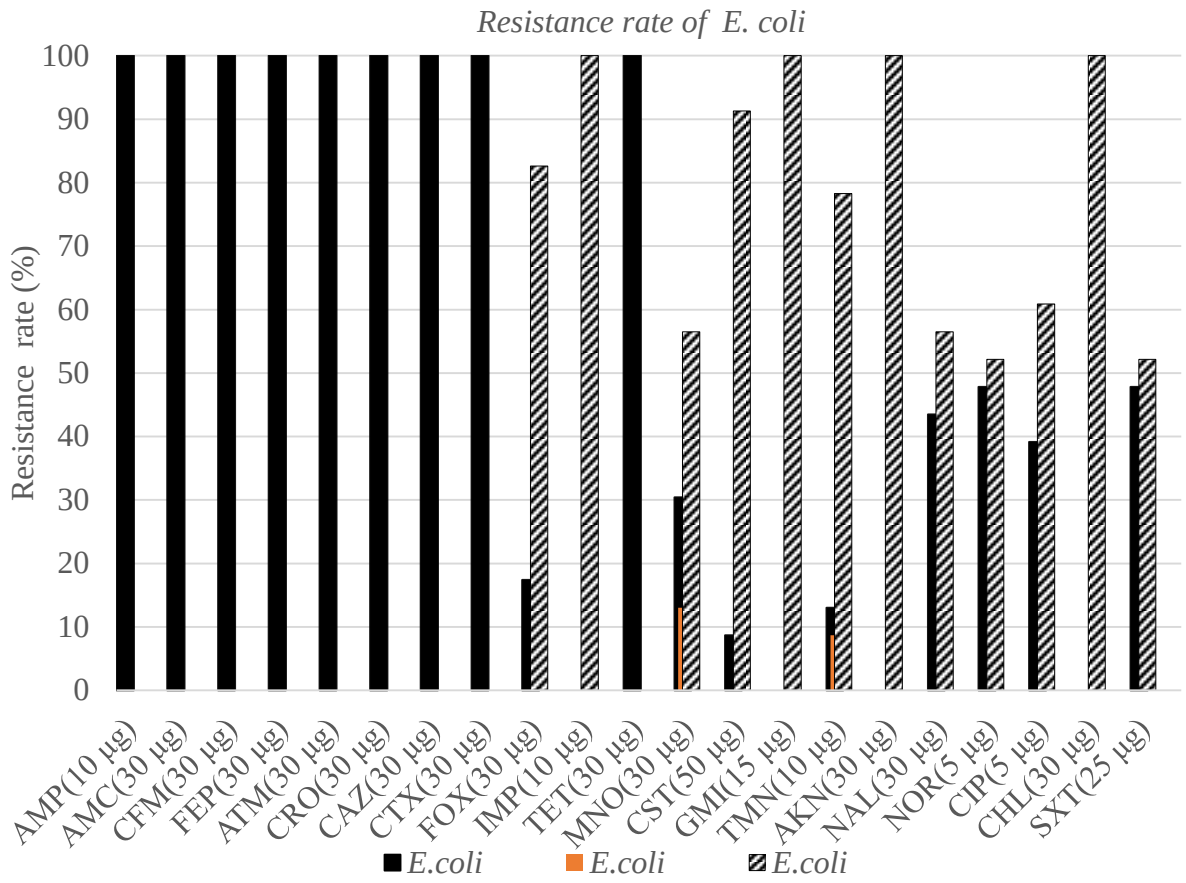

Figure 1. *Resistance rate of E. coli spp. strains to antibiotics*

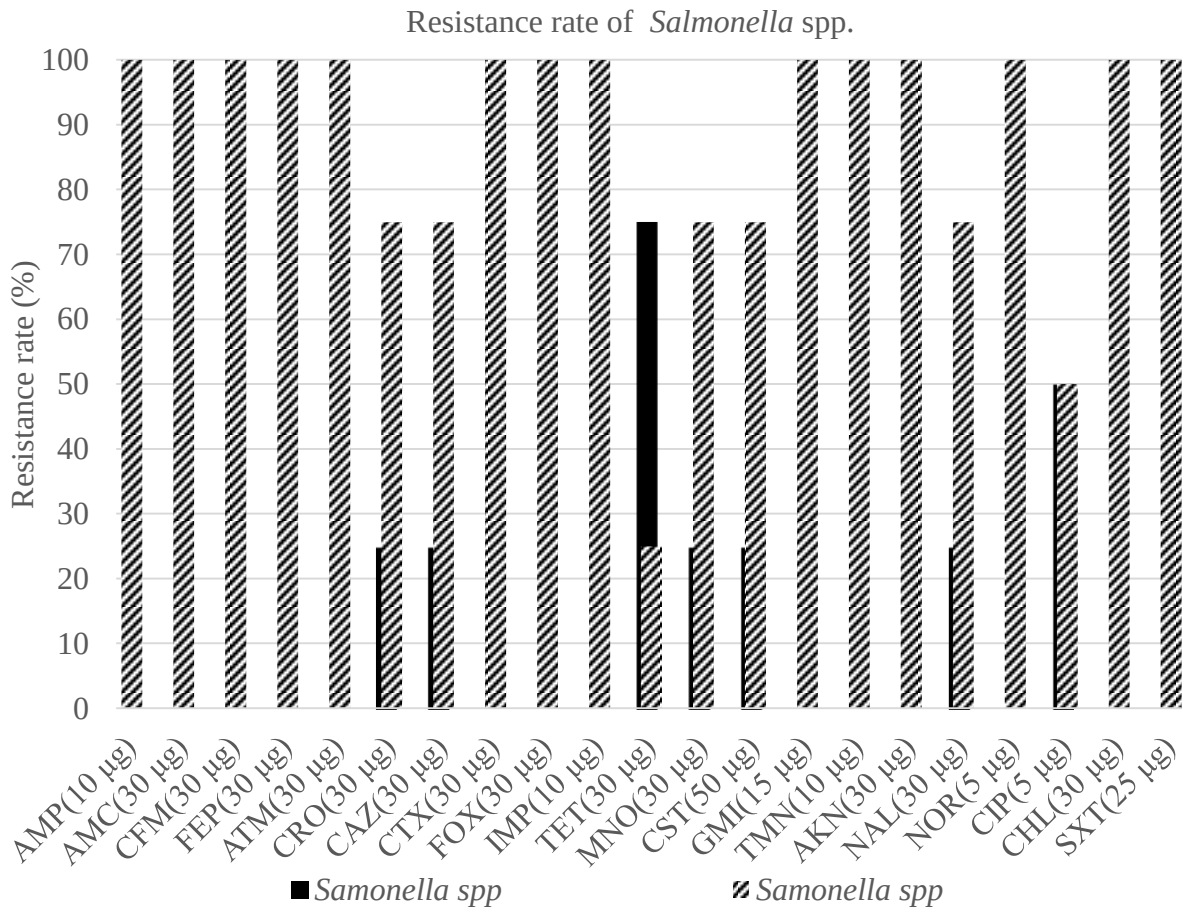

Figure 2. *Resistance rate of Salmonella spp. strains to antibiotics*

Tableau 1. *Profil de résistance des souches de E. coli isolées des bouses de bovins*

Strain N=23	AMP	AMC	CFM	FEP	ATM	CRO	CAZ	CTX	FOX	IPM	TET	MNO	CST	GMI	TMN	AKN	NAL	NOR	CIP	CHL	SXT
Disk	10µg	30µg	5µg	30µg	30µg	10µg	10µg	5µg	30µg	10µg	30µg	30µg	50µg	10µg	10µg	30µg	30µg	10µg	5µg	30µg	25µg
R+I N (%)	23 100	23 100	23 100	23 100	23 100	23 100	23 100	23 100	4 19,04	0	23 100	9 39,13	0 0	0 0	5 21,74	0 0	10 43,48	11 47,83	9 39,13	0 0	11 47,83
S N (%)	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	19 80,96	23 100	0 0	14 60,87	23 100	23 100	18 78,26	23 100	13 56,52	12 52,17	14 60,87	23 100	12 52,17

AMP *= Ampicillin,* ***AMC*** *= Amoxicillin + Clavulanic acid,* ***CFM*** *= Cefixime,* ***FEP*** *= Cefepime,* ***ATM*** *= Aztreonam,* ***CRO*** *= Ceftriaxone,* ***CAZ*** *= Ceftazidime,* ***CTX*** *= Ceftriaxone,* ***FOX*** *= Cefoxitin,* ***IMP*** *= Imipenem,* ***TET*** *= Tetracycline,* ***MNO*** *= Minocycline ,* ***CST*** *= Colistin, GMI = Gentamicin,* ***TMN*** *= Tobramycin,* ***AKN*** *= Amikacin,* ***NAL*** *= Nalidixic acid,* ***NOR*** *= Norfloxacin,* ***CIP*** *= Ciprofloxacin, CHL = Chloramphenicol,* ***SXT*** *= Trimethoprim / Sulfamethoxazole*

Tableau 2. *Profil de résistance des souches de Salmonella spp. isolées des bouses de bovins*

Strain N= 4	AMP	AMC	CFM	FEP	ATM	CRO	CAZ	CTX	FOX	IPM	TET	MNO	CST	GMI	TMN	AKN	NAL	NOR	CIP	CHL	SXT
Disk	10µg	30µg	5µg	30µg	30µg	10µg	10µg	5µg	30µg	10µg	30µg	30µg	50µg	10µg	10µg	30µg	30µg	10µg	5µg	30µg	25µg
R N (%)	0 0	0 0	0 0	0 0	0 0	1 25	1 25	0 0	0 0	0 0	3 75	1 25	1 25	0 0	0 0	0 0	1 25	0 0	2 50	0 0	0 0
S N (%)	4 100	4 100	4 100	4 100	4 100	3 75	3 75	4 100	4 100	4 100	1 25	3 75	3 75	4 100	4 100	4 100	3 75	4 100	2 50	4 100	4 100

N: number of strains, ***S****: sensitive,* ***R****: Resistant, Amoxicillin + clavulanic acid (****AMC****), Ceftazidime (****CAZ****), Aztreonam (****ATM****), Cefoxitin (****FOX****), Cefixime (****CFM****), Imipenem (****IPM****), Ampicillin (****AMP** *), Ceftriaxone (****CRO****), Cefepime (****FEP****), Ciprofloxacin (****CIP****), Nalidixic acid (****NAL****), Norfloxacin (****NOR****), Gentamicin (****GMN****), Amikacin (****AKN****), Tobramycin (****TMN****), Tetracycline (****TET****), Minocycline (****MNO****), Colistin (****CST****), Cefotaxime (****CTX****), Chloramphenicol (****CHL****), Sulfamethoxazole / Trimethoprim (****SXT****)*