Isolation of Antibiotic-Resistant Strains (*Salmonella* spp. and *E. coli*) from Wastewater from the Port-Bouët Slaughterhouse (Abidjan, Côte d'Ivoire)

Coulibaly Ibourahema, Coulibaly Kalpy Julien, Condé Fatoumata, Konaté Ibrahim, Koné Daouda

***Abstract*—The research presented in this memory was carried out at the Institute Pasteur in Côte d'Ivoire and carried out on *E. coli* and *Salmonella* spp. The objective of this study was to isolate *E. coli* and *Salmonella* spp. strains resistant to antibiotics from wastewater from the Port-Bouët slaughterhouse. From September 2017 to March 2018, 17 strains of *E. coli* and 7 *Salmonella* spp. were isolated in 63 samples of wastewater collected at the slaughterhouse of Port-Bouët. Their identification was performed according to conventional bacteriological tests. An antibiogram according to the disk diffusion method was carried out for 21 antibiotics used in human and veterinary medicine. The prevalence observed was 26.98% for *E coli* strains. and 11.11% for *Salmonella* spp. All strains of *E. coli* showed complete (100%) resistance to 3rd generation cephalosporins, aztreonam, amoxicillin + clavulanic acid, and ampicillin. However, family resistance rates of quinolones, aminoglycosides, sulfonamides and cyclins remain very high. The sensitivity of *E. coli* to imipenem and amikacin was 100%. *Salmonella* spp. strains, on the other hand, were resistant only to beta-lactam at lower levels compared to *E. coli* strains. coli. In-depth studies are needed to determine the resistance mechanisms of these bacteria..**

***Index Terms*- aminosides, antibiogramme, ß-lactamine, cyclines *Escherichia coli*, quinolones, *Salmonella* spp., sulfamides**

I. Introduction

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, slurry or via slaughterhouse effluents [1]. The slaughterhouse effluents correspond to all the liquid discharges produced on the slaughterhouse site, that is to say the water resulting from the slaughtering activity (process, washing) and the wastewater (sanitary). By their nature, these effluents are heavily loaded with bacteria [2]. 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 [3]-[5]. Ruminants, the main reservoir, would participate in the maintenance of the epidemiological cycle of pathogens [6], [7].

The intensive use of antibiotics for therapeutic, preventive or growth-stimulating purposes raises livestock, particularly cattle, in favor of resistant pathogens that can develop, survive and spread [8].

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.

According to WHO, 80% of the diseases that affect the planet's population are related to water pollution (WHO, 2004). In fact, most of the microorganisms that are the source of the great historical epidemics of water origin, have for normal habitat the intestines of the man and certain warm-blooded animals. This is why the control and monitoring of water quality, especially wastewater, seems more and more indispensable. The city of Abidjan is the economic capital of Côte d'Ivoire with a population of around 6 million inhabitants. Liquid discharges represent one of the main environmental problems facing the Port-Bouet lagoon, given the extent of pollution generated by various liquid discharges (industrial and household) and its impact on surface and underground water resources.

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

Coulibaly Ibourahema, Department of Biochemistry-Microbiology, UFR Agroforestry/University Jean Lorougnon Guédé, BP 150 Daloa, Ivory Coast
Coulibaly Kalpy-Julien, University Hospital Center (UHC) of Cocody, Unit of Chemistry and Microbiology of Environment
Condé Fatoumata, Department of Biochemistry-Microbiology, UFR Agroforestry/University Jean Lorougnon Guédé, BP 150 Daloa, Ivory
Konaté Ibrahim, Department of Biochemistry-Microbiology, UFR Agroforestry/University Jean Lorougnon Guédé, BP 150 Daloa, Ivory
Koné Daouda, Laboratory of Plant Physiology, Department of Biology, Faculty of Bioscience, Abidjan, Felix Houphouët-Boigny, University, BP V34 Abidjan 01, Ivory Coast

primary objective of this study is to isolate strains of Escherichia coli and Salmonella spp. antibiotic-resistant wastewater from the Port-Bouët slaughterhouse. For this purpose, there are two specific objectives that are primarily the isolation of strains of Escherichia coli and Salmonella spp. wastewater from the Port-Bouët slaughterhouse; and secondly the antibiotic resistance of these microorganisms (strains)..

II. Materials and Methods

A. Study site

The study was carried out at the slaughterhouse located in the municipality of Port-Bouët. Note that the abattoir Port-Bouët was built one year before the independence of Côte d'Ivoire and covers an area of 6 ha. It is the largest slaughterhouse in the city of Abidjan and Ivory Coast. In its management, the slaughterhouse of Port-Bouët consists of an administration; a cattle yard; a breeding park, a slaughter room and various points on the site where flaming heads, legs, tails and skins of animals. In addition, the slaughterhouse is not equipped with a pre-treatment or wastewater treatment system. The effluents are discharged directly through a pipeline system leading to the Ebrié's lagoon.

B. Sampling

The sewage samples were taken at eight (8) points on the site of the slaughterhouse of Port-Bouët. The points were selected according to a piping system that starts from the slaughter room until the water is discharged in the Ebrié Lagoon. These points were named from P1 to P8 as follows: P1 (slaughter room), P2 (exit from the slaughter room), P3 (in front of the 16 new administration), P4 (at the level of dwellings), P5 (point of buckling of heads of cattle and dwellings), P6 (outlet of the water from the pipeline), P7 (breeding stock) and P8 (contact with Ebrié Lagoon). The sampling sessions were performed once a week for 7 weeks between 9am and 10am; hours when the atmosphere at the slaughter room is more fluid. The samples were taken in sterile glass bottles of one liter (1L) and then transported using a cooler containing cold accumulators to preserve the samples. A total of 56 wastewater samples (7 per point) were collected from the Port-Bouët abattoir. Samples were immediately sent to the laboratory for analysis

C. Preparation of the bacterial suspension

A volume of 15 mL of wastewater from each sample was added to 15 mL vials and centrifuged at 3000 rpm for 5 minutes. After centrifugation, 1 mL of the pellet of each centrifuged sample was removed by a sterile pipette and added to a 9 mL volume of buffered peptone water (PTE) contained in a sterile test tube. The whole was homogenized (manually) for two (2) minutes and constituted our bacterial suspension.

D. E. coli search

The search for *E. coli* was carried out according to the method described by [9]. For this purpose, the Rapid E. coli medium was substituted for the Drygalski medium. Thus Rapid *E. coli* (ReC2) supplemented with 2 mg / L of Ceftazidime was used in this study.

E. Preparation of ceftazidime solution

2 mg of ceftazidime (antibiotic) was weighed and added to 5 mL of sterile distilled water to give an initial concentration of 0.4 mg / mL. The antibiotic solution was stored in a freezer at -20 ° C until use.

F. Preparation of ReC2 + Ceftazidime Medium for Isolation of E. coli

Following the preparation of Rapid E. coli (ReC2) agar according to the manufacturer's instructions, a volume of 20 mL of the medium was dispensed into sterile glass vials. Subsequently, a volume V_i of the initially prepared ceftazidime solution was added to each vial containing 20 mL of ReC2 agar. This volume was determined according to the following formula:

$$C_i . V_i = C_f V_f \qquad C_f = \frac{C_i . V_i}{V_f}$$

Ci: initial concentration of ceftazidime solution;
Vi: initial volume of ceftazidime solution; See: final concentration of Rapid *E. coli* agar; V_f : final volume of ReC2 agar. Thus, to obtain Rapid *E. coli* agar at a concentration of 2 mg / ml, a volume of 100 µl of the ceftazidime solution with a concentration of 0.4 mg / ml was added to each vial containing 20 ml of Rapid *E. coli*. After homogenization, the contents of each vial were poured into a petri dish.

G. Isolation of E. coli

The isolation consisted in inoculating the bacterial suspension on Rapid E. coli agar supplemented with ceftazidime (ReC2 + Ceftazidime) by the streak exhaustion technique. The seeded petri dishes were subsequently incubated at 44°C. for 24 hours. Presumptive colonies of violet-colored E. coli on agar were subsequently identified from the oxidase, catalase and reduced Leminor rack assays.

H. Isolation of Salmonella spp.

The search for strains of *Salmonella* spp. was carried out according to standard NF ISO 6579 relating to the search for salmonella in foodstuffs and environmental samples. It was carried out in three main steps namely first, the pre-enrichment step which consisted in incubating at 37°C, the bacterial suspension previously prepared for 24 hours. Then the enrichment step, a volume of 0.1 ml of the pre-enriched solution was seeded into 10 ml of Rappaport Vassiliadis. The whole homogenized was incubated at 44°C for 24 hours to form the enriched solution. Enrichment in a selective medium (Rappaport Vassiliadis) allowed the development of salmonella while delaying or inhibiting the growth of other microorganisms. Finally, the isolation step which consisted in seeding the enriched solution on the Hecktoen selective medium by the technique of streak exhaustion. The inoculated dishes were subsequently incubated at 37°C for 24 hours. After incubation, presumptive colonies of *Salmonella* spp. of translucent green

color with or without a black center were identified from oxidase, catalase and reduced Leminor rack tests.

I. Determination of the antibiotic resistance profile of strains of E. coli and Salmonella spp.

The determination of the antibiotic resistance profile of strains of *E. coli* and *Salmonella* spp. consisted in testing 21 disks of antibiotics belonging to the families of beta-lactams, aminoglycosides, cyclins, phenicols, sulfonamides and quinolones. The confirmed strains E. coli and Salmonella spp. following the reduced Leminor rack were purified on plain agar plate and resistance profile determined. For this purpose, an isolated agar isolated colony fragment was removed using a dropper pipette and suspended in physiological saline solution (10 ml). The well homogenized inoculum constituted the bacterial suspension. Turbidity measurement was performed using the 0.5 McFarland BioMereux Densimat.

The inoculum obtained was used to seed the Müller-Hilton agar (MH) previously cast in the Petri dishes. To do this, a sterile cotton swab was immersed in the inoculum. Excess liquid was removed by rotating the swab on the tube walls to prevent over-flooding of the agar. The swab was performed on the entire agar surface in three directions according to EUCAST / CA-SFM 2017.

Antibiotics (**Table 1**) were plated onto MH agar plates seeded using the disk dispenser (7 per dish). The dishes were subsequently incubated at 37°C for 24 hours. After incubation, the zones of inhibition were read using software, ADAGIO, to define the sensitive, intermediate or resistant categories of the isolated strains.

Table 1: Table of antibiotic's discs used

Families	Antibiotics tested	Abbreviations	Charge	Critical concentrations (mg/L)		Critical diameter's (mm)	
				S ≤	R >	S ≥	R<
β-lactams	Amoxicillin +Clavulanic acid	AMC	30 µg	8	8	19	19
	Ceftazidime	CAZ	30 µg	1	4	22	19
	Aztreonam	ATM	30 µg	1	4	26	21
	Cefoxitin	FOX	30 µg	8	16	19	15
	Cefotaxime	CTX	30 µg	1	2	20	17
	Imipenem	IMP	30 µg	2	8	22	16
	ampicillin	AMP	30 µg	8	8	14	14
	Ceftriaxone	CRO	30 µg	1	2	25	22
	Cefepime	FEP	30 µg	1	4	27	21
	cefixime	CFM	5 µg	1	1	17	17
Quinolones	Ciprofloxacin	CIP	5 µg	0,25	0,5	26	24
	Nalidixic acid	NAL	30 µg	16	16	19	14
	Norfloxacin	NOR	5 µg	0,5	1	22	19
Aminoglycosides	Gentamicin	GMI	15 µg	2	4	17	14
	Amikacin	AKN	30 µg	8	16	16	13
	Tobramycin	TMN	10µg	2	4	17	14
Cyclins	Tetracycline	TET	30 µg	4	8	19	17
	Minocycline	MNO	30 µg	4	8	19	17
Phenicols	Chloramphenicol	CHL	30 µg	8	8	17	17
Sulfonamides	Trimethoprim/sulfamethoxazole	SXT	25 µg	2	4	14	11
Others	colistin	CST	50 µg	2	2	15	15

III. Results

A. Isolation frequency

The results of the microbiological analyzes made it possible to determine the biochemical characteristics of the strains of *E. coli* and *Salmonella* spp. summarized in the following **table** 2

Table 2. Biochemical characteristics of strains of *E. coli* and *Salmonella* spp.

Tests	Gram	Ox	Cat	Urée	Ind	Glu	Lac	H_2S	Gaz	LDC	LDA	Man	Cit
E. coli	-	-	+	-	+	+	+	+/-	+/-	+	-	+	-
Salmonella	-	-	+	-	-	+	-	+/-	+/-	+	-	+	+

About of the 56 samples collected and analyzed, 17 strains of *E. coli* were isolated, a prevalence of 30.36% and 5 strains of *Salmonella* spp. a prevalence of 8.93% responded to these traits.

3.2. Difference of contamination according to the points

The diagrams below show the differences in contamination as a function of the sampling points. Indeed, points 1 and 7 respectively corresponding to the point of the slaughter room and the point near the breeding stock were the most contaminated.

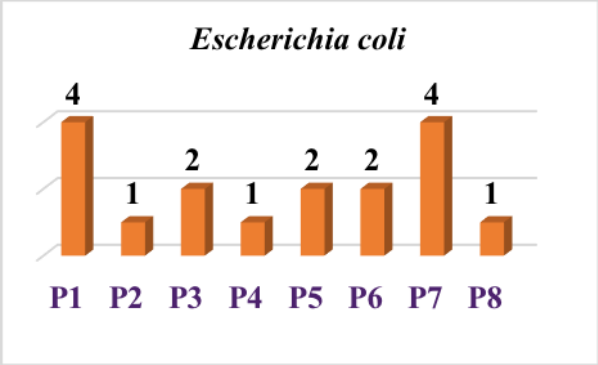

Figure 1. Difference in *E. coli* contamination by sampling points

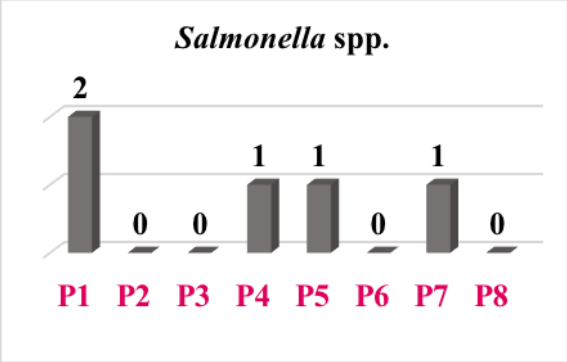

Figure 2: Difference in contamination with *Salmonella* spp. according to the sampling points

3.3. Antibiotic resistance profile of strains of E. coli and Salmonella spp.

The results showed that the *E. coli* strains isolated from the wastewater analyzed were all sensitive to imipenem (17/17 ie 100%). Regarding other antibiotics of the beta-lactam family, the strains showed complete resistance (17/17 ie 100%) to ceftazidime, ceftriaxone, amoxicillin + clavulanic acid, aztreonam, cefotaxime and ampicillin. The resistance rate still remains high for cefepime and cefixime (16/17 is 94.12%) and lower for cefoxitin with 5/17 or 20.41%. The resistance rates of aminoglycoside strains were lower with 3/17 or 17.65% for gentamicin and 6/17 for 35.29% for tobramycin. All *E. coli* strains were sensitive to amykacin (0/17 ie 0%). Regarding quinolones, resistance levels were 10/17, ie 58.82% for norfloxacin, 9/17 for 52.94% for nalidixic acid and 8/17 for 47.06% for ciprofloxacin. The cyclin resistance levels were high for tetracycline with 16/17 or 94.12% and a little less for minocycline with 9/17 or 52.94%. For the other antibiotics, a high level was obtained with Trimethoprim/Sulfa -methoxazole (15/17 ie 88.24%), a lower rate with chloramphenicol (3/17 or 17.65%) and a high low with colistin (1/17 ie 5.88%) (Figure 3).

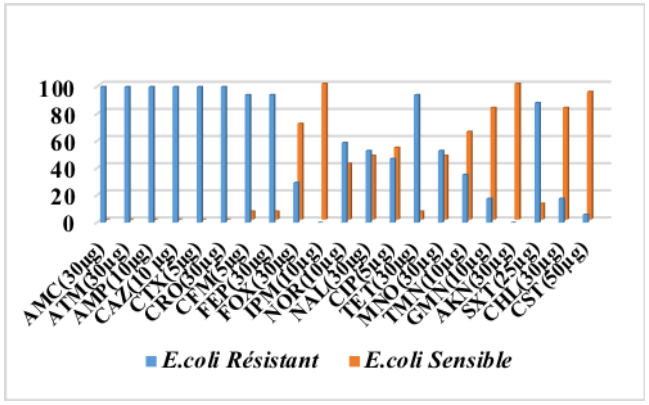

Figure 3: Resistance rate of *Escherichia coli* isolated according to antibiotics

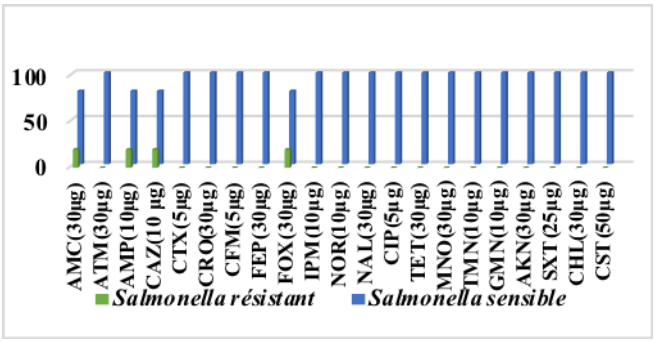

Figure 4: Resistance rate of *Salmonella* spp. isolated according to antibiotics

IV. Discussion

This study shows a high prevalence rate of *E. coli* strains (17/56 or 30.36%) in effluents from the Port-Bouët abattoir. The findings of [1] reveal a higher prevalence rate in slaughterhouse effluents (1.18%) compared with those obtained in urban effluents. These studies also demonstrated that 25% of the samples isolated in the slaughterhouse effluent were pathogenetic. The prevalence of Salmonella was also highlighted with a rate of 8.93%, results similar to those of [10] and [11] who respectively obtained Salmonella levels of 5% and 6.66%, respectively. %.

These results support the assumption that animals are reservoirs of *E. coli* and *Salmonella* spp. The antibiotic susceptibility profile of the 17 strains of E. coli isolated in our study shows a high resistance to the family of beta-lactams including third-generation cephalosporins, aztreonam and the group of penicillin (100%). These results do not agree with those obtained by [12], who reported lack of resistance to cefotaxime (0%), ceftazidime (0%) in 33 strains of *E. coli* isolated from purified raw water and Cultures in Morocco. These results can be explained by the high use of antibiotics

belonging to the beta-lactam family in the treatment of diseases within the Port-Bouët slaughterhouse. However, these strains remained sensitive to amykacin and imipenem (0%). These results agree with those obtained by [13], (36.4% for amykacin and 100% for imipenem). One might think that these antibiotics are not used in the treatment of diseases at the slaughterhouse of Port-Bouët therefore occupy a place of choice in the therapeutic treatment of severe infections with multi-resistant bacteria. Moreover, this sensitivity of isolated *E. coli* strains remains quite close to the ESBL isolated by [14]. This suggests chances of finding broad-spectrum beta-lactam-producing strains (ESBL). For quinolones, the resistance levels of E. coli were 47.06% for ciprofloxacin, 52.94% for nalidixic acid and 58.82% for norfloxacin. These results are close to those of Fofana (2004) (54.44% with nalidixic acid). Quinolones are currently the most important group of antibiotics. Their interest is related to their low toxicity and especially to the absence of plasmid resistance [13]. Studies carried out by INRA researchers [15] and [16 have shown that strains of Escherichia coli, of animal origin, which are highly resistant to quinolones, have appeared by a significant modification of the target. For aminoglycosides, the most active molecule was tobramycin with 35.29% resistance. This result contrasts with that of [16] who achieved a 36.4% resistance rate for amikacin. This rate is still lower than that obtained in Turkey, which is 64% [17]. For other antibiotics, the study found a high level of resistance to trimethopine / sulfamethoxazole (88.24%) and the cyclin family; tetracycline (94.12%) minocycline (52.94%). Only colistin had good activity with 5.88% resistance.

The *E. coli* strains isolated from this study showed fairly high percentages of resistance to several families of antibiotics. Beta-lactams are currently the most important group of these antibiotics. On the contrary, these results showed that strains of Salmonella still remained sensitive (0%) to several families of antibiotics. However, Salmonella isolated in this study were resistant to one antibiotic of the family of beta-lactam antibiotics with a prevalence rate of 4/5 or 80%. These results contrast with those obtained by [8] who show a prevalence of 81.1% of strains of Salmonella spp. to an antibiotic and more. These strains showed multiple resistance (68.49%) to five antibiotics (ampicillin, trimethoprim, Trimethoprim sulphametoxazole, tetracyclines, sulfonamides). These percentages are comparable to those reported in other studies in France [15] and Ethiopia [18]. *E. coli* strains exhibited higher levels of resistance than Salmonella, as reported in the literature [19] [20]. It is likely that the isolated *E. coli* strains were under pressure from previous antibiotic therapy. The more a bacterial species or serotype is encountered in pathology, the higher the resistance frequencies [21].

The cross-resistance of ceftazidime-resistant strains of E. coli from the strain isolation stage was observed for all beta-lactams. Salmonella also showed cross-resistance to this same family of antibiotics. Cross-resistance indeed to a chromosomal origin and it concerns only antibiotics of the same family, therefore having a common site of action. Thus, resistance to an antibiotic may be accompanied by resistance to other antibiotics of the same family.

It is from this study that strains of E. coli and Salmonella spp. antibiotic-resistant respectively 7/17 (41.17%) and 1/5 (20%) were found close to contact with Ebrié's lagoon (stockyard). These strains released into the environment, therefore represent a risk to public health by contamination of surface wastewater. It should be noted that water from the Ebrié lagoon in direct contact with slaughterhouse effluent is used by the surrounding population for fishing, market gardening, which poses a real threat to the health of the populations.

In sum, the importance of bacterial resistance observed in this study reflects the previous use of antibiotics, particularly beta-lactams, in cattle farms in the Port-Bouët slaughterhouse for curative or prophylactic purposes. A better control of the evolution of the resistance of the bacteria to the antibiotics is only possible by disciplining the use of these products simultaneously in the man and the animal

V. Conclusion

The results of this study show that effluent discharges from the Port-Bouët slaughterhouse contribute to the maintenance of the environmental cycle of antibiotic-resistant strains E. coli and Salmonella spp. The environment would therefore constitute a reservoir of multi-resistant strains where many gene exchanges would occur leading to the emergence of new clones pathogenic and / or resistant to humans. Antimicrobial resistance is a constantly evolving phenomenon that affects the entire bacterial world and all families of therapeutic antibiotics. This situation makes it difficult to choose effective measures to limit the erosion of the antibiotic spectrum. To prevent the spread of resistance, the abattoir of Port-Bouët should set up a network to monitor the resistance of bacteria of animal origin. It would also be interesting to select a more representative number of strains of Salmonella and Escherichia coli, and to repeat this work to evaluate the impact of environmental spread of resistant bacteria on public health. An analysis of the strains by molecular biology tools could allow on the one hand to acquire information on the resistance phenotypes and on the other hand, to distinguish within the bacterial populations, the clonal diffusion phenomena of resistant strains or the resistance gene transfers.

References

[1] Diallo A. A. Pathogenic and antibiotic-resistant *Escherichia coli* in effluents of human and animal origin: Prevalence and characterization before and after treatment, Doctoral Thesis in Microbiology, Graduate School of Biology-Health-Biotechnology, Toulouse III University - Paul-Sabatier, Toulouse, France, 2013, p. 204.

[2] Medad, Order of 30 April 2004 on the requirements applicable to facilities classified for the protection of the environment subject to authorization under heading No. 2210 "slaughter of animals". NOR: DEVP0430124A. Official Journal of the French Republic. 141: 11034.

[3] Jakobsen L., Sandvang D., Hansen L.H., Bagger-Skjøt L., Westh H., Jørgensen C., Hansen D.S., Pedersen B.M., Monnet D.L., Frimodt-Møller N., Sørensen S.J., & Hammerum A.M. Characterization, dissemination and persistence of gentamicin resistant *Escherichia coli* from a Danish university hospital to the waste water environment. International environment. 34., 2008, pp. 108-15

[4] Prado T., Pereira C.W. & Silva D.M. Detection of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in effluents and sludge of a hospital sewage treatment plant. Letters Applied Microbiology., 2008, 46, pp. 136-41.

[5] Yang C. M., Lin M.F., Liao P.C., Yeh H.W., Chang B.V., Tang T.K., Cheng C., Sung C.H. & Liou M.L. Comparison of antimicrobial resistance patterns between clinical and segregation in a regional hospital in Taiwan. Letters Applied Microbiology. 48: 2009.pp. 560-565

[6] Holler C., Koschinsky S. & Witthuhn D. Isolation of enterohaemorrhagic *Escherichia coli* from municipal sewage. Lancet, 1999,353, p. 2039

[7] Vernozy-Rozand C., Montet M.P., Lequerrec F., Serillon E., Tilly B., Bavai C., Ray-Gueniot S., Bouvet J., Mazuy-Cruchaudet C. & Richard Y. Prevalence of verotoxinproducing *Escherichia coli* (VTEC) in slurry, farmyard manure and sewage sludge in France. Applied Microbiology Journal. 93: 2002. pp. 473-8.

[8] Fofana A. (2004). Antibiotic resistance of Escherichia coli strains isolated from broiler meat in Senegal, Mémoire DEA, Animal Production, Dakar (EISMV), 2004, p 43.

[9] Elhamzaoui S., Benouda A., Allali F., Abouqual R & Elouennass M. 2009. Antibiotic susceptibility of isolated Staphylococcus aureus strains in two university hospitals in Rabat, Maroc. Med Mal 2009

[10] Dodo N. Prevalence of strains of *Escherichia coli* and isolated Salmonella in live cattle dung in the Port-Bouët livestock park, 2013.

[11] Kouakou A. Serotypes and antibiotic profile of strains of Salmonella spp. isolated cattle dung cattle park in the town of Port-Bouet, 2013, (Abidjan, Ivory Coast).

[12] N. Oubrim, Cohen N. & Hajjami K. Detection of Fecal Enterococci and *Escherichia Coli* Resistant to Isolated Antibiotics from Purified Raw Waters and Crops. European Journal of Scientific Research, Morocco.2012, pp. 453-461

[13] Bennett P. M. Plasmid Encoded Antibiotic Resistance: Acquisition and Transfer of Antibiotic Resistance Gene in Bacteria, British Journal Pharmacology .153 (1), 2008, pp. 347-357.

[14] Alekshun M.N. & Levy S.B. The mar regulon: multiple resistance to antibiotics and other toxic chemicals, Trends Microbiology. 7., 1999, pp. 410-3.

[15] Andersson D.I. & Hughes D. Antibiotic resistance and its cost: is possible to reverse resistance, Nature Review Microbiology. 8., 2010, pp. 260-271.

[16] Sanders P., Gicquel M., Humbert F., Perrin-Guyomard A. & Salvat G. Plan for surveillance of antibiotic resistance in indicator bacteria isolated from the intestinal flora of pigs and poultry from 1999-2001, Bulletin Academy Veterinary, France, 155 (3/4): 2002, pp. 267-276.

[17] Guessennd N., Kacou-N'douba A., Gbonon V., Yapi D., Ekaza E., Dosso M. & Courvalin P. Prevalence and resistance profile of broad-spectrum beta-lactamase-producing enterobacteria (ESBL) in Abidjan Côte d'Ivoire from 2005 to 2006. Journal of Pharmaceutical and Biological Sciences. 9 (1), 2008, pp. 63-70.

[18] Eski F., G. Ozer & Balci I. Investigation of the frequency of extended spectrum beta-lactamases and antibiotic resistance in clinical isolates of Escherichia coli and Klebsiella spp., Mikrobiyol Bulteni, 41 (3), 2007, p. 447-5.

[19] Tibaijuka B., Molla B., Hildebrandt G., Kleer J. & Salah W. Anti-Microbial Resistance to Salmonella Isolated from Retail Raw Chicken Meat and Poultry Offal. Animal Health Production Africa Newsletter. 50 (2), 2002, pp. 86-95.

[20] Leclerc H., Izard D., Husson M. O., Watter P. & Jakubczak E. General Microbiology, New. Edition. -Paris: Doin Edition, 1983, p. 369.

[21] Sante Canada. Use in Canada of antimicrobials in food animals: implications for human health. Report of the Advisory Committee on the Use of Antimicrobials in Animals and Consequences for Resistance and Human Health., 2002

[22] Martel J. L. Epidemio-surveillance of the antimicrobial resistance of pathogenic bacteria in animals. Epidemiology Animal Health. 29., 1996, pp. 107-120.

[23] J. Jones. (1991, May 10). Networks (2nd ed.) [Online]. Available: http://www.atm.com

[24] (Journal Online Sources style) K. Author. (year, month). Title. *Journal* [Type of medium]. Volume(issue), paging if given. Available: http://www.(URL)

[25] R. J. Vidmar. (1992, August). On the use of atmospheric plasmas as electromagnetic reflectors. *IEEE Trans. Plasma Sci.* [Online]. *21(3).* pp. 876—880. Available: http://www.halcyon.com/pub/journals/21ps03-vidmar

.