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Resistance Profile of Strains of *Escherichia coli* and *Salmonella* sp. Isolated from Raw Cow Milk Sold in the Department of Vina in Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author BA supervised and was involved in writing the original draft as well. Author MAD was involved in the conceptualization. Author WM carried out the survey. Author TV was involved in the development of the methodology. Author AD managed the software and provided monitoring and author NA supervised and validated the manuscript revision and editing. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Antimicrobial resistance, defined by the WHO as "resistance of a micro-organism to an antibiotic to which it was previously sensitive", results from the ability of the bacterium to withstand the attack of antibiotics. This resistance is a major factor in the risk of therapeutic failure and the

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spread of multidrug-resistant strains from animals to humans. The present study was aimed to assess the antibiotic resistance of strains of Escherichia coli and *Salmonella* sp. Isolated from cattle farms in the Department of Vina, Cameroon.
Place and Duration of Study: The study involved 30 strains of *Salmonella* (05) and *Escherichia coli* (25) isolated from 60 samples of raw cow milk, collected from July to August 2021 in Vina, Adamawa Province. The samples were each made up of 15 ml of milk, taken aseptically from cattle farms and sales depots.
Methodology: Antibiotic susceptibility of 25 strains of *Escherichia coli* and 5 *Salmonella* sp. strains was wanted, according to the standard Mueller-Hinton agar diffusion method 16 antibiotic discs.
Results: The diameters of the inhibition zones formed showed fairly high resistance rates, 87.5% for *Salmonella* sp. and 75% for *E. coli.* Bacterial strains are all resistant to the Penicillin, Macrolides and Diaminopyrimidines tested. Good sensitivity was observed with levofloxacin (100%), cefixime (82.14%) and gentamycin (78.60%).
Conclusion: The importance of these resistances reflects the intensive previous use of antibiotics in livestock farming as growth-promoting additives.

Keywords: Antimicrobial resistance; Salmonella sp.; Escherichia coli; antibiogram, raw milk.

1. INTRODUCTION

The discovery and use of antibiotics in the last century has led to a change in medical history, improving the life-threatening outcome for many patients with infectious diseases (Vincent, & Le Bâcle, 2015).

But the emergence of antibiotic-resistant bacterial strains has ended the optimism generated by the discovery of effective antibiotics for the treatment of infections (Barika, El H., & Boussaidi, 2019). This resistance is now a serious public health problem around the world (Kahn, 2016).

Antimicrobial resistance, defined by the WHO as “resistance of a micro-organism to an antibiotic to which it was previously sensitive”, results from the ability of the bacterium to withstand the attack of antibiotics. It occurs when the micro-organism mutates or acquires a resistance gene (Vincent, & Le Bâcle, 2015).

The WHO now says that antibiotic resistance is one of the most serious threats to global health. Similarly, it predicts that by 2050, antibiotic-resistant infectious diseases will be the leading cause of death by disease (WHO, 2016). This resistance is a major factor in the risk of therapeutic failure and the spread of multidrug-resistant strains (Sambe-Ba, et al., 2013).

The development of antibiotic resistance in food-borne bacteria (*Salmonella enterica*, Campylobacter sp., etc.) is also a concern (Acar & Rostrel, 2001). The molecular characterization of antibiotic resistance mechanisms has shown the existence of mobile genetic structures that play a very important role in the dissemination of resistance: plasmids, integrons and transposons (Perugini, et al., 2015).

Antibiotics are widely used in prophylactic livestock systems, or as feed additives or growth factors for animals (Zinedine, et al., 2007, Ben-Mahdi & Ouslimani, 2009). The dairy sector appears to be the most antibiotic-consuming sector, compared with the breastfeeding sector (Cazeau, et al., 2010, Sulpice, et al., 2017). This type of use induces changes in the digestive flora of animals, leading to the emergence of resistant strains (Fabre, et al., 2000, Gysi, 2006).

Adamawa is one of the three main livestock regions in Cameroon, along with the northern and northwestern provinces. However, there is little data on the resistance of strains of *Salmonella* sp and *E. coli* bovines in this region and particularly in the department of Vina.

Faced with the multiple concerns about the presence of pathogenic and antibiotic-resistant bacterial strains in animals and the lack of a national antimicrobial resistance monitoring system, we were led to evaluate the antibiotic sensitivity of *Escherichia coli* and *Salmonella* sp strains. Isolated dairy cows in the Vina Department.

2. MATERIALS AND METHODS

2.2 Sampling

The study involved 30 strains of *Salmonella* (05) and *Escherichia coli* (25) isolated from 60 samples of raw cow milk, collected from July to August 2021 in Vina, Adamawa Province. The samples were each made up of 15 ml of milk, taken aseptically from cattle farms and sales depots (Fig. 1).

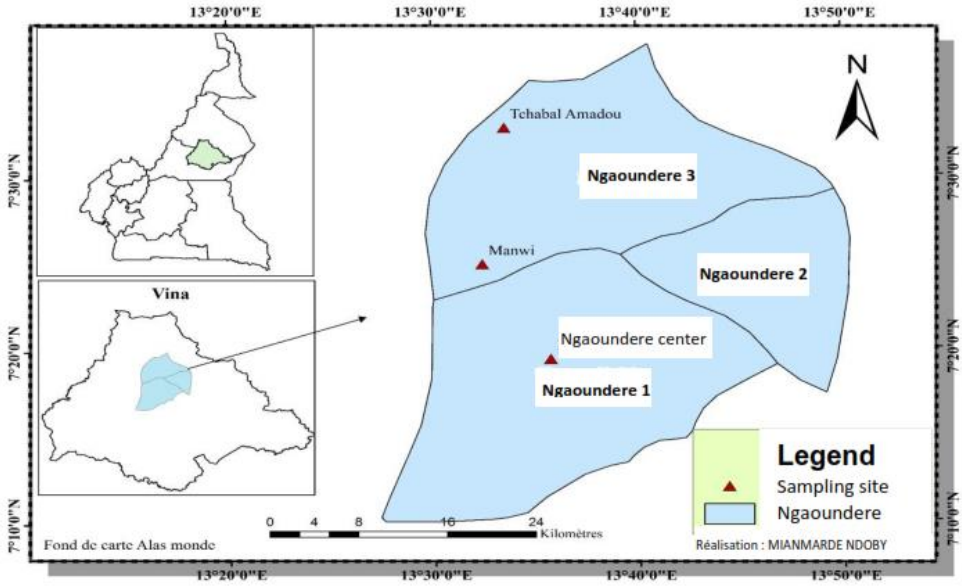

Fig. 1. Sampling point location map

2.2 Bacteriological Analyses

The isolation of *Salmonella* sp strains. and *Escherichia coli* a met the NF/EN ISO 6579-1:2017/Amd.1:2020 reference methods and macConkey Sorbitol agar seeding. The sensitivity of the strains was tested using the Mueller-Hinton method as recommended by the French Microbiology Society's Committee on Susceptibility (CA-SFM/EUCAST, 2016).

These analyses were carried out at the Microbiology laboratory of the Institute for Agricultural Research for Development (IRAD) in Wakwa, N'Gaoundere.

2.2.1 Detection and identification of strains of Salmonella sp. and Escherichia coli

2.2.1.1 Salmonella sp.

The samples, pre-enriched to 1/10 with buffered peptonated water were maintained 30 mn at room temperature for revivification and then incubated 18 to 20 hours between 34 and 38°C. After incubation, the suspension obtained was enriched by inoculation of 0,1 ml of pre-enrichment in 10 ml of Rappaport Vassiliadis Soya (RVS) medium and 1 ml in 10 ml of Mueller-Kaufmann Tetrathionate medium enriched with novobiocine (MKTTn). The enrichment products were used to inoculate the selective agar and BGA SS gelosized media incubated then 24 hours at 37°C. Isolated colonies, characteristic of *Salmonella* (opaque, translucent or transparent with usually a black center) were re-dosed to new SS Agar and BGA for first purification and 24 h later on nutrient agar for second purification (ISO, 2002).

2.2.1.2 Escherichia coli

A box of MacConkey Sorbitol agar was seeded with the dilution 10^{-1}following the quadrant method and incubated for 24 hours at 37°C. After incubation, the characteristic colonies (colorless or beige) were re-seeded to MacConkey Sorbitol for purification (Mainil, 2003).

The pure and isolated *Salmonella* and *E. coli* colonies obtained were successively subjected to biochemical confirmatory tests by inoculation of the slope Kligler-Hajna agar and then by inoculation of galleries® API 20E (Bio-Mérieux).

2.2.1.3 Research on the susceptibility of bacterial strains to antibiotics

Fresh colonies of the isolated and identified bacterial strains were suspended in a tube of distilled water and calibrated to 0.5 MacFarland turbidity. The resulting bacterial suspension was used to flood the entire surface of the Mueller Hinton agar medium. Absorbent paper discs or antibiotic-impregnated bio-discs were subsequently placed equidistant from each other on the medium with a pair of pliers, lightly pressing to ensure contact with the agar. In order to obtain a pre-diffusion of the antibiotics, the boxes were kept for 15 min at the laboratory

Table 1. Antibiotic Discs used

Antibiotic families	Antibiotics	Codes	Charge (µg)
-Lactamine (Penicillin)	Amoxicillin + clavulanic acid	CMA	30
	Amoxicillin	AML	10
	Ampicillin	AM	10
	Oxacilline	OX	1
	Penicillin G	P	10
	Cloxacillin	CX	5
-Lactamine (Cephalosporins)	Ceftriaxone	CRO	30
	Céfépime	FEP	30
	Cefixime	CFM	5
	Cefotaxime	CTX	30
Phoenicicolous	Chloramphenicol	C	30
Quinolones	Levofloxacin	LVX	5
Aminosides	Gentamycine	GM	10
Macrolides	Erythromycine	E	15
	Amphotericin	AMB	20
Diaminopyrimidines	**Trimethoprim**	SXT	1,25

temperature before being incubated 24 hours at 37°C.

After incubation, inhibition zones centered around the antibiotic discs appeared. The diameter of these areas, measured using a caliper and interpreted according to CASFM criteria (CA-SFM/EUCAST, 2016).

The analysis covered 16 antibiotic discs (BIORAD), which met both their concentrations and controls to WHO standards (Table 1).

2.3 Statistical Analyses

The database was generated, entered and processed using Access, Excel and Word (Microsoft office corporation 2021). The data collected were subsequently analyzed using STATGRAPHICS centurion 17.1.06 and SPSS at 5%.

3. RESULTS AND DISCUSSION

Antibiotic resistance rates observed *for E. coli* and *Salmonella* sp*.* are shown in Figs. 1 and 2 respectively. These fairly high levels are 75% for E. coli*,* and 87.5% for *Salmonella sp.*

For resistance of *E. coli* strains (Fig. 2), the highest levels were observed with Ampicillin, Penicillin G, Cloxacilline and Erythromycin (100%), Amoxicillin, Amphotericin, Trimethoprim and Cefepime (96%), Ceftriaxone and Amoxicillin + clavulanic acid (84%) and finally Chloramphenicol (68%). The lowest rates were reported for levofloxacin (00%), cefixime (12%) and gentamycin (16%).

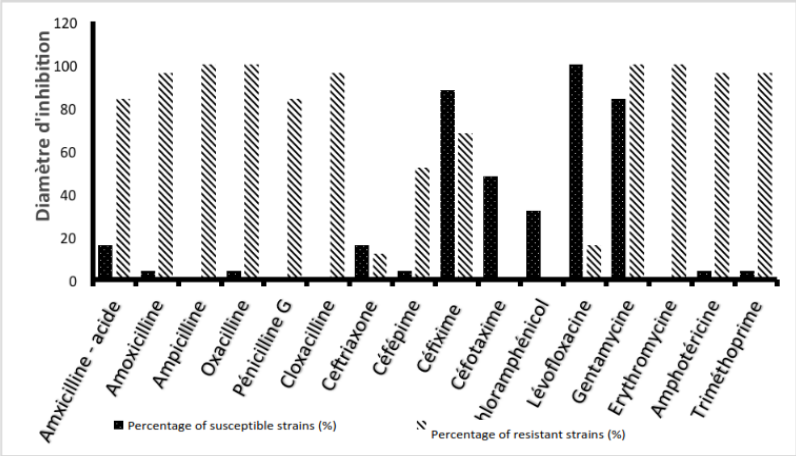

Fig. 2. *E. coli* strain resistance rates

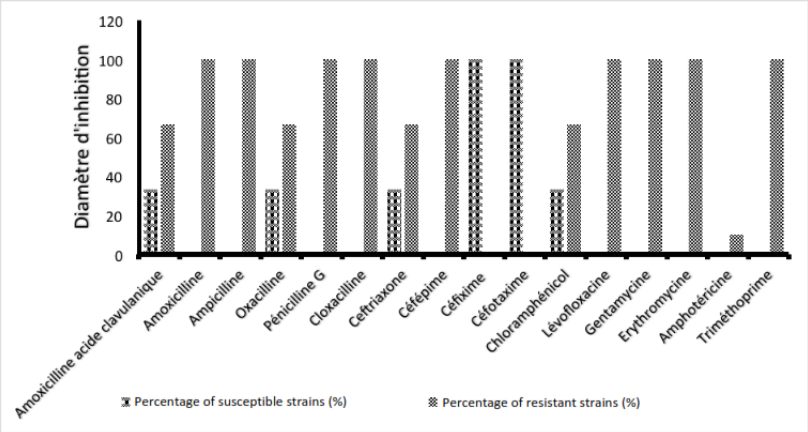

Fig. 3. Resistance rates of *Salmonella sp*

For the *Salmonella* strains, we can note a very good sensitivity (100%) to two of the 16 antibiotics tested, chloramphenicol and levofloxacin. Medium sensitivity (33.33%) to 6 antibiotics, Amoxiciline+clavulanic acid, Oxacillin, Ceftriazone, Cefixime, Gentamycin and Trimethoprim. The highest resistance rates were observed with amphotericin, erythromycin, cefotaxime, cefepime, cloxacillin, penicillin G, ampicillin (65 to 100%) (Fig. 3).

Isolates of *Salmonella* and *E. coli* forms showed multiple resistance to the antibiotics tested. This multiresistance concerns antibiotics commonly used in veterinary medicine, especially β-Lactamine, the associations Amoxicillin + clavulanic acid, macrolides as well as 3rd and 4th generation cephalosporins (Ceftriaxone and Cefepime) and Trimethoprim are also used in the treatment of various bacterial infections in humans. Our results are similar to those of Solomakos et al., (2009), Savadogo et al. (2010), Bagré et al. (2014) which revealed that some strains of *Salmonella* sp. and *E. coli* isolates of dairy products in Greece, Burkina Faso and other countries were resistant to amoxicillin, amoxicillin-clavulanic acid, Trimethoxazole, erythromycin, tobramycin, chloramphenicol and sulfonamides.

However, the strains of *Salmonella* sp et from *E. coli* isolates in this study remain sensitive to Chloramphenicol and Levofloxacin (100%). Which is close to the results obtained by Ashosi et al., (2018) in DR Congo.

The relatively high rates of resistance observed are not very surprising, given the risk factors and the uncontrolled use of antibiotics in the farms surveyed. It should be noted that in the natural environment, bacteria can host resistance genes derived from antibiotic use in animals (Health Canada, 2005). It is also important to note that the enterobacteria have an obvious ability to acquire and exchange resistance factor genes, and the intestinal flora provides a tremendous opportunity for the collection of genetic information between bacteria (Van, et al., 2005).

In terms of resistance to β-lactam, this profile is commonly found in enterobacterial bacteria with hyperproduced cephalosporinase and carbapenemases phenotypes. These results corroborate those of Roger et al., (2013) from their work on raw milk in the city of Maroua.

Enterobacteriaceae generally use different mechanisms of resistance to β-Lactams. These may include antibiotic permeability disorders, which prevent the antibiotic from penetrating the bacteria, efflux systems that allow antibiotics to be evacuated from the bacteria, or modification of the bacterial target of the antibiotic. But most frequently, they are enzymes destroying beta-lactamases (Guillot, 1989, Vora & Auckenthaler, 2009, Eddair, et al., 2024). All gram-negative bacteria are chromosomally mediated β-lactamase enzymes. This also implies that resistance to cephalosporin may confer resistance to other β-lactams such as penicillin (Guillot, 1989, Vora & Auckenthaler, 2009, Eddair, et al., 2024).

For Sala et al., (2012), breeders and veterinarians are more likely to use antibiotics of the β-lactam family to treat bovine infections. The high resistance of bacilli to gram-negative β-lactam could thus be justified by the fact that

antibiotics in this family are easily available and affordable.

The sensitivity of these bacteria to aminosides may be related to the fact that aminosides, particularly amikacin and to a lesser extent gentamicin, remain among the most active antibiotics on all enterobacteria (Benhiba, et al., 2015, Landecker, 2021). This sensitivity may also be related to the fact that these antibiotics are all administered by injectable route, which requires a certain technicality and therefore an additional cost for their use. Farmers will be much less likely to use them for the treatment of infections in their livestock. The selection pressure exerted by these antibiotics is lower, so the likelihood of resistance is less (Guillot, 1989), (Benhiba, et al., 2015, Landecker, 2021). According to Benhiba et al., (2015), the rational use of this molecule would be linked to this low resistance rate. The effects of selection pressure can also be cited for Chloramphenicol and Levofloxacin; the molecules of this family are less well known and therefore less used (Benhiba, et al., 2015, Landecker, 2021).

Indeed, the phenomenon of resistance in breeding is aggravated by the fact that very often farmers mix antibiotics with food as a substitute, and this without any rules or control (Ungemach, et al., 2006). The result is the selection of many strains resistant to several families of antibiotics at once, which can contaminate animals and humans and make all treatments by antibiotics difficult or impossible (Okechukwu, et al., 2020).

4. CONCLUSION

The bacterial strains isolated show multiple and fairly high resistance to the antibiotics tested. The highest resistance rates were observed with the most commonly used and known antibiotics such as β-Lactams. The importance of these resistances reflects intensive previous use in livestock. Such evolution of resistant bacteria is most often linked to the molecular bases and resistance mechanisms that condition the epidemiology of antimicrobial resistance. It is therefore urgent to regulate the use of antibiotics in livestock, as a growth factor, preventive or curative. This should make it possible to promote a rational use of antibiotics and to monitor the evolution of antimicrobial resistance in humans and animals in a coordinated way.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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