



(RESEARCH ARTICLE)



Antibiotic resistance of salmonella strains isolated from poultry products (eggs and organs) from markets and slaughter areas in the Bamako district

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Abstract

The aim of this study was to determine the antibiotic resistance of Salmonella strains isolated from poultry products from Bamako's markets and slaughter areas. It involved the analysis of 64 Salmonella strains isolated from poultry eggs and organs. The antibiogram profile of these strains was determined using the Kirby-Bauer agar diffusion method and the recommendations of the Comité de l'antibiogramme de la société française de microbiologie (CA-SFM). This work resulted in high rates of resistance to imipenem (93.75%), followed by kanamycin (84.37%) and doxycycline (67.18%). In addition, low levels of resistance were observed to gentamicin (12.5%) and flumequine (26.56%). Sequencing of the complete genome of the isolated strains and the use of bioinformatics tools made it possible to obtain certain genes conferring antibiotic resistance, namely marB, marA, marC, marR, mdtG, mdtQ, folA, ksgA, rarD and STM169. Some of these genes are multi-resistant to antibiotics (marB, marA, marC).

Keywords: Antibiotic Resistance; Salmonella; Poultry Products; Bamako.

1. Introduction

Food-borne diseases are a major cause of morbidity worldwide. Salmonellosis is the leading cause of food-borne illness [7,15]. The main sources of human contamination are products of avian origin, such as eggs and poultry meat [7]. The fight against avian salmonellosis in Mali is mainly based on the abusive use of antimicrobial products, in this case antibiotics [16,17]. These molecules block the growth of bacteria or destroy them. However, recent years have seen the spread of antimicrobial resistance among non-typhoidal *Salmonella* isolates in humans [8]. This antibiotic resistance represents a threat to human health and a real public health problem [16, 11, 5,6]. Certain strains of multi-resistant *Salmonella* of animal origin are suspected of having acquired their resistance genes before being transmitted to humans via the food chain [7, 14]. This multi-resistance poses a twofold risk to livestock production, through treatment failure, and to humans, through ingestion of food derived from these products. Despite the implementation of the national action plan to combat antimicrobial resistance in Mali in 2019, antibiotic resistance continues to rise.

2. Materials and methods

Biological material used consists of guinea fowl and hen eggs and organs.

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2.1. Study site

This study was carried out in the poultry markets and slaughter areas of the Bamako district.

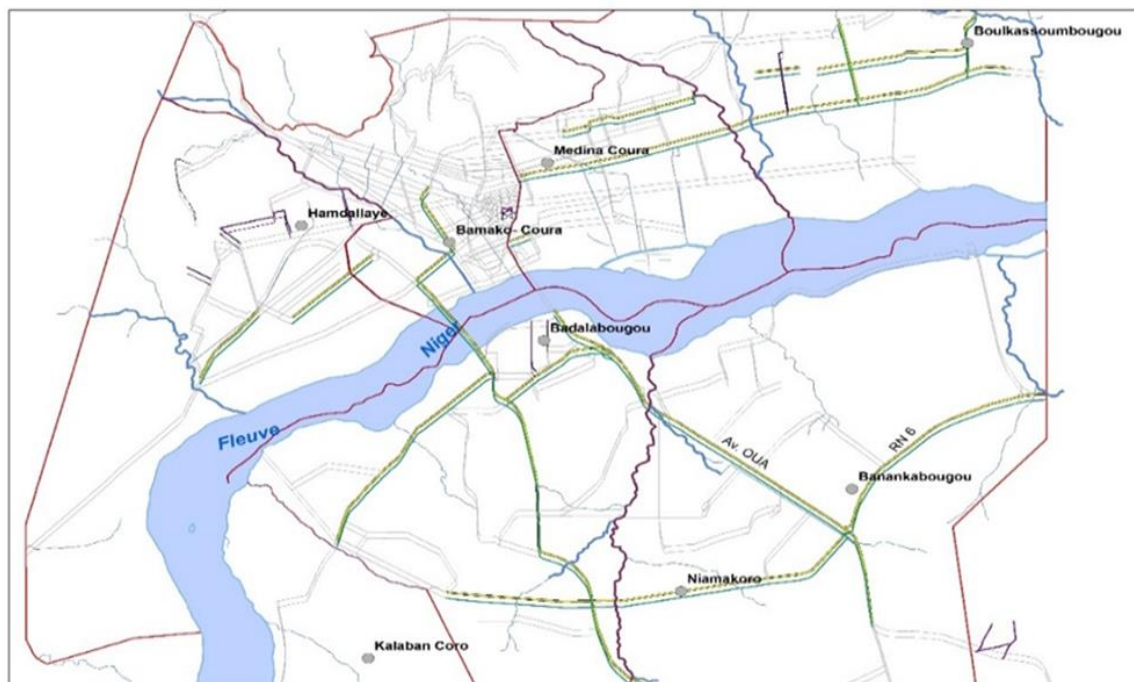


Figure 1 Geographical location of sampling sites

Source: National Institute of Geography of Mali

2.2. Salmonella testing

Samples submitted to the laboratory were analyzed in three stages. Firstly, each swab was soaked in peptone water, then incubated at 37°C for 24 hours. Organs were ground beforehand, diluted to 10% in peptone water (1 ml/g of ground material weighed) and then incubated for 24 h. Next, the tetrathionate broth was inoculated with one milliliter of pre-enrichment culture on peptone water, then incubated at 37°C for 24 h. Finally, from the cultures obtained on the enrichment media, selective media, such as Mac Conkey and Salmonella Shigella agars, were inoculated, then incubated at 37°C for 24 h. Characteristic Salmonella colonies, appearing colorless with a black center on Salmonella-Shigella and Mac Conkey agars, were observed. Pure cultures obtained on tryptone soy agar were used for classical biochemical tests or API systems.

2.3. Determination of antibiotypic profile

Antibiotic resistance of strains was determined using the Kirby-Bauer agar diffusion method and the recommendations of the Comité de l'antibiogramme de la société française de microbiologie (CA-SFM). Resistance profiles to eight antibiotics present in drug complexes commonly used in modern poultry farming was determined. The antibiotic disks used are : Gentamycin (10µg), Flumequine (30µg), Kanamycin (30µg), Tetracycline (30µg), Doxycycline (30µg), Streptomycin (10µg), Colistin (50µg), Imipenem (10µg). For this purpose, each strain was plated on tryptone soy agar and incubated at 37°C for 24 h to obtain pure cultures. From colonies of the 24 h culture, a turbid suspension was made in two milliliters of physiological water (NaCl 85%) at Mac Farland's 0.5 scale. Inoculum was obtained by emulsifying 100 µl of this suspension in 10 ml of physiological water. Mueller Hinton agar in Petri dishes was streaked with swabs soaked in bacterial suspension. Discs of the eight antibiotics were placed on a disc dispenser. The plates containing the discs were left at room temperature in a hood for 30 min, then incubated for 24 h at 37°C. The diameter of the zones of inhibition was determined and the values were interpreted after comparison with those given by the manufacturers.

2.4. Research into antibiotic resistance genes

After extraction and purification of DNA from isolated salmonella strains, they were sent to our partners in the genomics laboratory for whole genome sequencing (WGS) and exom sequencing (WES). Using bioinformatics tools such

as Gene scan and Ensembl, this sequence data was used to locate resistance genes and *Salmonella* coding regions in the sequence.

3. Results

Testing for salmonella in poultry eggs and organs resulted in the isolation of 64 salmonella strains. Determination of the antibiotypic profile of these Salmonella strains yielded the results shown in Table 1 below.

Table 1 Antibiotic resistance profile of isolated salmonella strains

antibiotics	Resistant (%)	Sensitive (%)
Gentamycin (10µg)	12,50	87,5
Flumequine (30µg)	26,56	73,44
Kanamycin (30µg)	84,37	15,63
Tetracycline (30µg)	39,06	90,94
Doxycycline (30µg)	67,18	32,82
Colistin (50µg)	43,75	56,25
Streptomycine (10µg)	51,56	48,44
Imipenem (10µg)	93,75	6,25

Analysis of this table shows that the highest rates of resistance were observed with the following antibiotics: imipenem 93.75%, followed by kanamycin 84.37% and doxycycline 67.18%. However, low resistance rates were observed with gentamicin 12.5% and flumequine 26.56%.

Table 2 Resistance genes identified from isolated serotypes

<i>Salmonella</i> Serotypes	<i>S enteritidis</i>	<i>S typhimurium</i>	<i>S indiana</i>	<i>S hadar</i>	<i>S enfantis</i>
Antibiotic resistance genes	marB mdtG mdtQ	folA , ksgA marB ,marA marC, rarD STM169	marB	marB mar R	marR marB

Analysis of Table 2 shows the presence of 10 resistance genes. The marB gene was the most predominant. It was identified in most of the Salmonella serotypes isolated.

3.1. Primary structure

MKMLFPA**L**PG**L**LLIASG**Y**GIAEQ**T**LLPVAQ**N**SRD**V**ML**L**PC**V**GD**P**PN**D**L**H**PS**V**NSDK**S**DE**L**GV**P**Y**N**D**Q**HL

Figure 2 FASTA-formatted sequence of the conserved functional domain of the marB gene^[19]

In this figure, conserved amino acids appear in color. This domain has conserved residues in all enterobacteria, demonstrating its conservative and stable role in the bacterial genome.

4. Discussion

The present study obtained a salmonella resistance rate to tetracycline of 39.06%, which is lower than those obtained by Hamadou et al^[11] in Chad, who obtained 51.22%, and Coulibaly et al^[5] in Côte d'Ivoire, who obtained 100% resistance to tetracycline. This could be explained by the fact that on poultry farms, many farmers use antibiotics to prevent and cure disease. Tetracyclines and doxycyclines are commonly used molecules in veterinary therapeutics, and belong to the family of antibiotics most widely used in humans. The resistance of salmonella strains to imipenem is

thought to be due to the production of carbapenemases that inactivate them. The high sensitivity of isolated *Salmonella* strains to gentamycin is due to the fact that this molecule is rarely used in the poultry industry in Mali, due to its extremely high cost. Resistance to kanamycin, colistin and streptomycins is certainly due to their misuse in growth promoters. Most poultry growth promoters marketed in Bamako contain colistin and streptomycin. The discovery of *Salmonella* isolates resistant to flumequine is of great concern, as this family of antibiotics is used as a last resort in the treatment of severe human salmonellosis.

The sequencing of the complete genome of the *Salmonella* strains isolated and the use of bioinformatics tools enabled the present study to obtain certain resistance genes. Among the genes isolated, the *marB* gene was found in most of the *Salmonella* serotypes isolated. This could be explained by the fact that bacteria are constantly exchanging genetic material, which favours the growth and survival of the bacteria that contain it, as well as that of their neighbors. This same observation was made by Hassan et al [12] and Haritha Routh [13]. We also noted that the *marB*, *marA*, *marC* and *marR* genes confer multiple antibiotic resistance on *Salmonella*. This is a real public health problem. Our observation is similar to that made by Sidibé et al [16] and Toko et al [18]. The protein motif determines which amino acids are most important for protein function, based on their size and frequency. The present study has shown that any mutation in the amino acids of these conserved residues can lead to denaturation of the protein's function. Targeting key amino acids in the domain whose modification leads to loss of protein structure and function may be a technique or solution in the search for antibiotics against multi-resistant bacteria (finding a consensus epitope), which may be a therapeutic target for which simulated protein-protein interaction or docking may be envisaged for future studies.

5. Conclusion

Salmonella strains isolated from poultry products (eggs and organs) from Bamako markets and slaughterhouses are resistant to most commonly used antibiotics. In addition, the presence of resistance genes in isolated *Salmonella* strains confirms the high level of antibiotic resistance. Some of these resistance genes confer multi-antibiotic resistance on *Salmonella* strains.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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