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# Prevalence and Antibiotic Resistance of *Salmonella* spp. and *Staphylococcus aureus* Isolated from Broiler Chicken Meat in Modern and Traditional Slaughterhouses of Morocco

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**ABSTRACT**

Handling and consuming contaminated meat can lead to food poisoning and the acquisition of antibiotic resistance genes. *Staphylococcus aureus* (*S. aureus*) and *Salmonella* spp. are the most isolated bacteria from broiler chicken meat, leading to serious foodborne diseases. The present study aimed to evaluate the presence and antibiogram profiles of *Salmonella* spp. and *S. aureus* strains in poultry meat purchased from modern and traditional poultry slaughterhouses in Morocco. Foodborne pathogens, such as *Salmonella* spp. and *S. aureus*, were isolated from poultry meat using standard methods and then confirmed by biochemical tests (coagulase, catalase, oxidase, motility and API 20E for further biochemical identification) and an immunological test (serotyping test). The antibiogram of the isolates was determined using the agar diffusion method and interpreted according to the criteria of performance standards for antimicrobial susceptibility testing of the Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA. A total of 540 poultry meat samples were collected and treated (360 poultry meat samples from traditional slaughterhouses and 180 poultry meat samples from modern slaughterhouses), out of which 15.92% were *S. aureus* positive and 7.40% were *Salmonella* spp. positive. In traditional poultry slaughterhouses, the prevalence rates of *Salmonella* spp. and *S. aureus* were 11.11% and 20.55%, respectively. In contrast, *Salmonella* spp. was not detected in poultry samples of modern poultry slaughterhouses, and the prevalence of *S. aureus* was 6.66%. All *S. aureus* and 97% of *Salmonella* spp. isolates were found resistant to at least one antibiotic, while 86% of *S. aureus* and 30% of *Salmonella* spp. showed resistance to more than three antibiotics. The obtained results of the present study confirmed that broiler chicken meat purchased from traditional poultry slaughterhouses was mainly contaminated by *Salmonella* spp. and *S. aureus*, indicating a major public health risk in Morocco. Therefore, considerable efforts should be made to apply appropriate hygiene practices.

**Keywords:** Antimicrobial resistance, Broiler chicken meat, Modern slaughterhouses, *Salmonella*, Prevalence, *Staphylococcus aureus*, Traditional slaughterhouses

## INTRODUCTION

Foodborne diseases (FBD) are pathological cases caused by the ingestion of food containing biological, chemical, or physical hazards. They affect both developed and developing countries and have an impact on public health and economy (Akbar and Anal, 2013). Several pathogenic bacteria can cause FBD, among which *Salmonella* spp. and *Staphylococcus aureus* (*S. aureus*) are the most common pathogenic bacteria in animal-source foods (Rortana et al., 2021).

Non-typhoidal Salmonella is a significant contributor to severe invasive illnesses in adults, children, and immunocompromised individuals in Africa (El-Tayeb et al., 2017). The invasive *Salmonella* spp. infections can affect several organs, such as the digestive tract, endothelial surfaces, pericardium, meninges, lungs, joints, bones, genitourinary tract, resulting in bacteremia, meningitis, osteomyelitis, or septic arthritis and sometimes even death (WHO, 2015). Of *Salmonella* spp. pathogens, *Salmonella enteritidis* and *typhimurium* are the two most commonly reported serotypes of human foodborne in most parts of the world (WHO, 2015). On the other hand, *S. aureus* infections can also range from minor skin problems to severe infections (MDH, 2010). It produces many toxins, including staphylococcal enterotoxins, which can cause FBD (ANSES, 2022).

In Morocco, there has been a progressive rise in collective food poisoning cases in recent years, indicating that cases have almost doubled in 9 years (from 866 in 2008 to 1631 in 2017, FISA, 2022a). Agents responsible for this issue have only been identified in 20% of cases, with 20% of *Salmonella* and 10% of *S. aureus* (DELM, 2018). Poultry meat is



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among the vectors responsible for these foodborne infections (Lundén et al., 2003; Prakash et al., 2005). As reported recently, consumption of poultry meat in the Moroccan kingdom has increased significantly over the last two decades, from 8.9 kg/year in 2000 to 19.3 kg/year in 2021, an increase of 117% (FISA, 2022a). Thus, poultry meat production, particularly broiler chicken meat in the formal sector, has experienced a progressive growth of 162% from 2000 (200,000 tons) to 2021 (525,000 tons, FISA, 2022a).

Another serious challenge facing humanity is antibiotic resistance. The prevalence of antibiotic-resistant foodborne pathogens is also increasing due to their excessive use in human and animal treatments (Akbar and Anal, 2013). Moreover, the determinants of antibiotic resistance can be transferred to other pathogenic bacteria, which can compromise the treatment of serious bacterial infections, and thus, constitutes a major threat to public health (Adesiji et al., 2011). This is the case with *Salmonella* strains resistant to antibiotics in most countries (Duc et al., 2019). Effectively, it has been reported in developed countries that this increased resistance in *Salmonella* spp. has a zoonotic origin, and the bacteria present in food animals acquire resistance before being transmitted to humans through the food chain (Threlfall, 2002; Andoh et al., 2016). Moreover, in poultry production, antibiotics are commonly used as Growth promoters at sub-therapeutic doses and obviously as treatment (FISA, 2022b). Therefore, chickens and chicken meat may contain antibiotic-resistant strains and serve as a vehicle for disseminating antibiotic-resistant strains to humans and the environment (Duc et al., 2019).

Notably, broiler chicken slaughter is carried out in formal and informal sectors in Morocco. The formal sector concerns approved industrial poultry slaughterhouses (Modern slaughterhouses), while the informal one represents the traditional slaughterhouses in the neighborhoods commonly known as "RYACHATE", which covers 80% of the Moroccan market (FISA, 2022b). However, the deplorable hygiene conditions of most traditional slaughterhouses present a threat to public health (FISA, 2022b). Therefore, in recent years, considerable efforts have been made to rationalize this sector and replace these traditional slaughterhouses with local, low-capacity poultry slaughterhouses that can meet the required hygiene standards by improving the legal framework. Given these conditions, the main objective of the present study was to evaluate the prevalence of *Salmonella* spp. and *S. aureus* contaminations in broiler chicken meat (neck skin, breast, and thigh) purchased from modern and traditional poultry slaughterhouses. This current study will help determine the prevalence and antibiotic resistance of the above-mentioned bacteria and discuss the major causes of bacterial contaminations during slaughtering.

## MATERIALS AND METHODS

### Ethical Committee Approval

All animal procedures in the present study were carried out following the Hassan II Agronomic and Veterinary Institute of Rabat and Moroccan Ministry of Agriculture recommendations, which are in accordance with international ethical standards (European Union Directive 2010/63/EU) legislation and ARRIVE (Animal Research Reporting of *in vivo* Experiments) guidelines.

### Sample collection

This study was carried out from July 2020 to February 2021 and concerned broiler chicken meat samples (the neck skin, breast, and thigh) from traditional and modern poultry slaughterhouses. Samples were randomly taken from the cities in Morocco, namely Casablanca, Mohammedia, Benslimane, Bouznika, and Rabat.

Regarding the informal sector, a total of 120 broiler chickens (Cobb 500) were purchased and slaughtered at four traditional slaughterhouses per city (Casablanca, Mohammedia, Benslimane, Bouznika, and Rabat) during the summer and winter seasons of the study period. While for the formal sector, a total of 60 broiler chickens in sealed and labeled trays from supermarkets (from modern poultry slaughterhouses) were purchased during the summer period. Once the samples were purchased, they were put in sterile collection bags and transferred to the laboratory of avian pathology at the Hassan II Agronomy and Veterinary Medicine Institute in Rabat, Morocco, in an isothermal box at 4°C. After that, chickens were sampled aseptically from the neck skin, thigh, and breast. Finally, 360 samples from the informal sector (Two seasons of Summer and Winter), and 180 samples from the formal sector (Summer season only) were collected.

### Isolation and identification of bacteria

The samples were analyzed separately for each bacterium. *Salmonella* spp. was isolated according to the International Standard ISO 6579, 2002. The 10 g of chicken samples (neck skin, thigh, breast) were transferred to water peptone buffer (CM 0509 Oxoid, Oxoid LTD, Basingstoke, Hampshire, England) and incubated at 37°C for 18-24 hours. Thereafter, 0.1 ml of the pre-inoculated water peptone buffer was transferred to Rappaport-Vassiliadis Soja (RVS, BK148HA Biokar diagnostics, Zac de Ther, France) and incubated at 42°C for 24 hours. A loopful of RVS was transferred to Xylose Lysine Deoxycholate agar (BK058HA Biokar diagnostics, Zac de Ther, France) and incubated at 37°C for 24-48 hours (ISO, 2002).



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All the isolated bacteria were identified based on their morphology, color, shape, and color change of culture media. They were also strained using Gram stains and examined with a light microscope x100 (OPTIKA B-151, ITALY) using oil immersion. Furthermore, biochemical tests of coagulase test (6BR0020, Biokar diagnostics, Zac de Ther, France), catalase test (1870 SOLVAPUR), oxidase test (MICROBAT Oxoid LTD, United Kingdom), and motility test were carried out on each isolate. This diagnosis was also confirmed by API 20E and serotyping test according to the Kauffmann-White scheme using a slide agglutination test with *Salmonella* polyvalent O and H antisera according to Diagnostic Pasteur, Paris, France, for *Salmonella* detection (WHO, 2007).

Regarding staphylococcal detection, the ISO 6888-1: 1999 standard was used. The 25 g from each sample of neck skin, breast, and thigh was placed in a sterile bag in 225 mL of water peptone buffer. The suspension was then homogenized using the Stomacher to obtain a stock suspension titrated at 1/10. A series of dilutions down to $10^{-5}$ was carried out from the stock solution at $10^{-1}$ by taking 1 mL each time added to 9 mL of distilled water in a test tube (ISO, 1999).

The prepared Petri dishes were inoculated with 0.1 mL of different dilutions with a sterile glass rake in Baird Parker's selective medium (BK055HA Biokar diagnostics, Zac de Ther, France) with egg yolk and potassium tellurite (3554205Bio-Rad Marnes-la-Coquette, France). The final preparations were incubated at 37°C for 24 to 48 hours. At the end of the incubation period, the suspect colonies of the *S. aureus* were black and shiny, surrounded by a halo of lightening of the medium, and were confirmed using two tests, including the DNAse test (CM0321 Oxoid, Oxoid LTD., Basingstoke, Hampshire, England) and the coagulase test (6BR0020, Biokar diagnostics, Zac de Ther, France, Papanicolas et al., 2014).

**Antimicrobial susceptibility test**

Antimicrobial susceptibility of *Salmonella* spp. and *S. aureus* tests were determined by Kirby-Bauer diffusion method using Mueller-Hinton agar and seven antibiotics discs for *Salmonella*: nalidixic acid (30 µg), gentamicin (15 µg), trimethoprim/sulfamethoxazole (1,25/23.75 µg), cefoxitin (30 µg), kanamycin (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), and 6 antibiotics discs for *S. aureus*, namely erythromycin (15 µg), trimethoprim/sulfamethoxazole (1,25/23.75 µg), tetracycline (30 µg), kanamycin (30 µg), streptomycin (25 µg), ampicillin (10 µg). All the discs were purchased from Oxoid LTD, England.

The pre-incubated (24 hours) cultures of *Salmonella* spp. and *S. aureus* were diluted in sterile normal saline to McFarland standards of 0.5 and then were inoculated onto the Mueller-Hinton agar surface agar (Bk048HA Biokar diagnostics zac de ther BEAUVAIS-France), where the antibiotic discs were placed, then the isolates were incubated at 37°C for 18-24 hours. The clear area around each antibiotic disc was measured in millimeters then the results were interpreted according to performance standard criteria for antimicrobial susceptibility testing by the Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA (CLSI, WEINSTEIN, 2018).

**Statistical analysis**

In order to analyze the results, three statistical methods were used. The chi-square ($\chi 2$) test was used to treat the descriptive analysis part as the prevalence study. The $p < 0.05$ was considered statistically significant. Multiple Correspondence Analysis (MCA) was employed to analyze the choice of exposure to the different tested risk factors. These methods aim to reduce the dimensions of the data tables to represent associations between individuals and between variables in small dimensions. The Cross-sectional study was used to answer the research questions of this experiment and therefore attempted to determine whether there was a relationship between various risk factors (season, sector type) and bacterial contaminations.

The ($\chi 2$) and MCA tests were realized by IBM SPSS version 24.0.0.0 statistical software. The Cross-sectional study was done by EPI INFO version 7.2.5.

## RESULTS

**Descriptive analysis**

Of 540 collected samples, the findings revealed that 86 were positive for *S. aureus* (15.92%) and 40 were positive for *Salmonella* spp. (7.40%), of which 27/40 (67.5%) were identified as *Salmonella enteritidis* while 13/40 (32.5%) were *Salmonella* Pullorum *and Salmonella* Gallinarum. In the traditional slaughterhouses, the prevalence of *Salmonella* spp. was 40/360 (11.11%), while the prevalence of *S. aureus* was 74/360 (20.55%), (Table 1).

Given that the effect of season on the prevalence of both *Salmonella* spp. and *S. aureus* were insignificant ($p > 0.05$), the data related to both seasons in the analysis of the effect of sector type on the prevalence of bacteria were pooled. In the formal sector, no *Salmonella* spp. was detected in poultry samples, and 12/180 (6.66%) of *S. aureus* was identified (Table 1).



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In the informal sector, it was found that out of all contaminated samples (114 samples), 8.7% of the broiler chicken meat samples were positive for both *Salmonella* spp. and *S. aureus.* The prevalence of *Salmonella* spp. was 19/180 (10.56%) during the winter, and 21/180 (11.67%) during the summer, while *S. aureus* 30/180 (16.67%) were positive during the winter and 44/180 (24.44%) during the summer (Table 2). In this study, the prevalence of *S. aureus* was significantly high in the neck skin, compared to the thigh and the breast ($p < 0.05$). However, the prevalence of *Salmonella* spp. was significantly higher in the thigh samples than in the neck skin and breast ($p < 0.05$, Table 3).

According to the Chi-2 test, the sample types and the prevalence of the bacteria studied were significantly related ($p < 0.05$). Concerning antibiotic resistance susceptibility, among all 40 *Salmonella* spp. isolates, 80% showed resistance to tetracycline, 57.50% to ciprofloxacin, 27.50% to kanamycin, 25% to nalidixic acid, and 5% to trimethoprim-sulfamethoxazole, while the lowest resistance of the isolates (2.50%) was against cefoxitin and no resistance to gentamycin (Table 4).

Regarding the antimicrobial susceptibility and resistance profiles of all *S. aureus* isolates, out of a total of 86 isolates, 47 showed resistance to erythromycin and 44 to streptomycin representing 54.65% and 51.16% of the total isolates, respectively. In contrast, only 1.16% showed resistance to trimethoprim-sulfamethoxazole. The highest resistance rate was against ampicillin, tetracycline, and kanamycin (100%, 81.40%, and 74.42%, respectively, Table 5).

The results revealed that 97.5 % of *Salmonella* spp. isolates showed resistance to at least one antibiotic, while 30% showed resistance to more than three antibiotics. However, all *S. aureus* isolates showed resistance to at least one antibiotic, and 86% showed resistance to more than three antibiotics (Graph 1, Table 6).

**Table 1.** Prevalence of *Staphylococcus aureus* and *Salmonella* spp. in informal and formal sectors

| | | Tested samples | Positive samples (%) | | p-value | |
|---|---|---|---|---|---|---|
| | | | ***Salm*[1]** | ***Staph*[2]** | ***Salm*** | ***Staph*** |
| Sector | Formal | 180 | 0 | 12 (6.66) | $p < 0.05^*$ | $p < 0.05^*$ |
| | Informal | 360 | 40 (11.11) | 74 (20.55) | | |

* Signifiant effet ($p < 0.05$). [1] *Salmonella* spp. [2] *Staphylococcus aureus*

**Table 2.** Prevalence of *Staphylococcus aureus* and *Salmonella* spp. in the informal sector

| | | Tested samples | Prevalence (%) | | p-value | |
|---|---|---|---|---|---|---|
| | | | ***Salm*[1]** | ***Staph*[2]** | ***Salm*[1]** | ***Staph*[2]** |
| Period | Summer | 180 | 21 (11.67) | 44 (24.44) | $p > 0.05$ | $p > 0.05$ |
| | Winter | 180 | 19 (10.56) | 30 (16.67) | | |
| Sample type | Breast | 120 | 5 (4.17) | 5 (4.17) | $p < 0.05$ | $p < 0.05$ |
| | Thigh | 120 | 23 (19.17) | 29 (24.17) | | |
| | Neck skin | 120 | 12 (10.00) | 40 (33.33) | | |
| City | Benslimane | 72 | 1 (1.39) | 3 (4.17) | $p < 0.05$ | $p < 0.05$ |
| | Bouznika | 72 | 9 (12.50) | 36 (50.00) | | |
| | Casablanca | 72 | 5 (6.94) | 18 (25.00) | | |
| | Mohammedia | 72 | 15 (20.83) | 13 (18.06) | | |
| | Rabat | 72 | 10 (13.89) | 4 (5.56) | | |

[1] *Salmonella* spp, [2] *Staphylococcus aureus*

**Table 3.** Distribution of *Staphylococcus aureus* and *Salmonella* spp. per sample type in the formal and informal sector

| Sample types | Breast | | | Thigh | | | Neck skin | | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive | Negative | Total | Positive | Negative | Total | Positive | Negative | Total |
| *Staphylococcus aureus* | 6 | 174 | 180 | 29 | 151 | 180 | 51 | 129 | 180 |
| *Salmonella* spp. | 5 | 175 | 180 | 23 | 157 | 180 | 12 | 168 | 180 |

**Table 4.** Antimicrobial susceptibility of *Salmonella* spp. isolated from broiler chicken meat samples in the traditional slaughterhouses

| ***Salmonella* spp. positive samples** / **Antibiotics** | Resistant (%) | | Intermediate (%) | | Sensitive (%) | |
|---|---|---|---|---|---|---|
| Tetracycline | 32/40 | (80.0) | 6/40 | (15.0) | 2/40 | (5.0) |
| Ciprofloxacin | 23/40 | (57.5) | 7/40 | (17.5) | 10/40 | (25.0) |
| Kanamycin | 11/40 | (27.5) | 18/40 | (45.0) | 11/40 | (27.5) |
| Nalidixic acid | 10/40 | (25.0) | 10/40 | (25.0) | 20/40 | (50.0) |
| Trimethoprim-sulfamethoxazole | 2/40 | (5.0) | 2/40 | (5.0) | 36/40 | (90.0) |
| Cefoxitin | 1/40 | (2.5) | 0/40 | (0.0) | 39/40 | (97.5) |
| Gentamycin | 0/40 | (0.0) | 2/40 | (5.0) | 38/40 | (95.0) |



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**Table 5.** Antimicrobial susceptibility of *Staphylococcus aureus* isolated from broiler chicken meat samples in the traditional and modern slaughterhouses

| *Staphylococcus aureus* positive samples / Antibiotics | Resistant (%) | | Intermediate (%) | | Sensitive (%) | |
|---|---|---|---|---|---|---|
| Ampicillin | 86/86 | (100.00) | 0/86 | (0.00) | 0/86 | (0.00) |
| Tetracycline | 70/86 | (81.40) | 14/86 | (16.28) | 2/86 | (2.32) |
| Kanamycin | 64/86 | (74.42) | 0/86 | (0.00) | 22/86 | (25.58) |
| Erythromycin | 47/86 | (54.65) | 38/86 | (44.19) | 1/86 | (1.16) |
| Streptomycin | 44/86 | (51.16) | 0/86 | (0.00) | 42/86 | (48.84) |
| Trimethoprim-sulfamethoxazole | 1/86 | (1.16) | 4/86 | (4.65) | 81/86 | (94.19) |

**Table 6.** Comparison of the number of *Salmonella* spp. and *Staphylococcus aureus* resistant to up to 5 antibiotics at the same time.

| Number of Antibiotics | Resistant isolates of *Salmonella* spp. | | Resistant isolates of *Staphylococcus aureus* | |
|---|---|---|---|---|
| | Total samples | Percentage | Total samples | Percentage |
| **0** | 1 | 2.5% | 0 | 0% |
| **1** | 12 | 30% | 1 | 1% |
| **2** | 15 | 37.5% | 11 | 13% |
| **3** | 10 | 25% | 22 | 26% |
| **4** | 2 | 5% | 37 | 43% |
| **5** | 0 | 0% | 15 | 17% |
| **Total** | **40** | **100%** | **86** | **100%** |

The antibiotics used for *Salmonella* spp. contained Nalidixic acid, Gentamicin, Trimethoprim/sulfamethoxazole, Cefoxitin, Kanamycin, Ciprofloxacin, Tetracycline; For *Staphylococcus aureus*: Erythromycin, Trimethoprim/sulfamethoxazole, Tetracycline, Kanamycin, Streptomycin, Ampicillin.

**Multiple correspondence analysis**

In MCA, the engaged factors were given two dimensions, which summarize the information given by the set investigated variables. Thus, Cronbach's Alpha value was 80%, indicating that all the variables measure the same construct. Figure 1 measures the trend of results for each variable. A grouping of variables neck skin and *S. aureus* was noticed, which confirmed a correlation by reflecting the high rate of *S. aureus* isolated in this sample. This goes to all correlations, resulting in a grouping represented in the graph.

Figure 2 is labeled by the identifier of each observation (sample). In the current study, cases were numbered from 1 to 540. On the graph, there was a grouping of all observations on one side except for 12 observations (all in the formal sector) and followed some different modalities. The two dimensions of the domain (dimension 1 with 26.5% of variance and dimension 2 with 11.68% of variance) depended on the projection model used. In this study, the choice was made on dimension 1, which best explained the direction of exposure between positives - negatives and the determining factors (Figures 1 and 2). Concerning Figure 3, it grouped correlating variables into four groupings, including *S. aureus* and neck skin, formal and informal sectors, *Salmonella* spp. and thighs, as well as summer and winter seasons. This was in accordance with the results obtained.

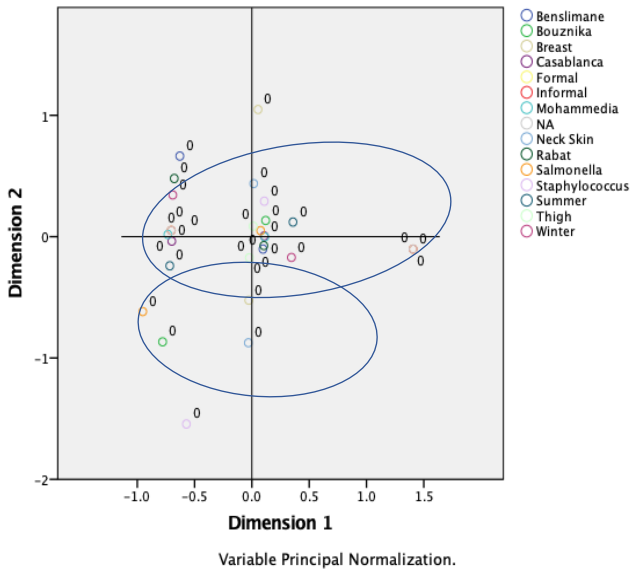


**Figure 1.** Projection of variables on the factorial axes

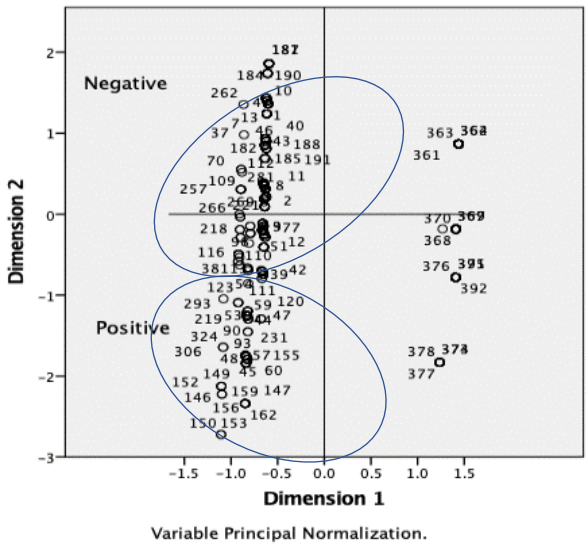


**Figure 2.** Projection of positives and negatives on the factorial axes



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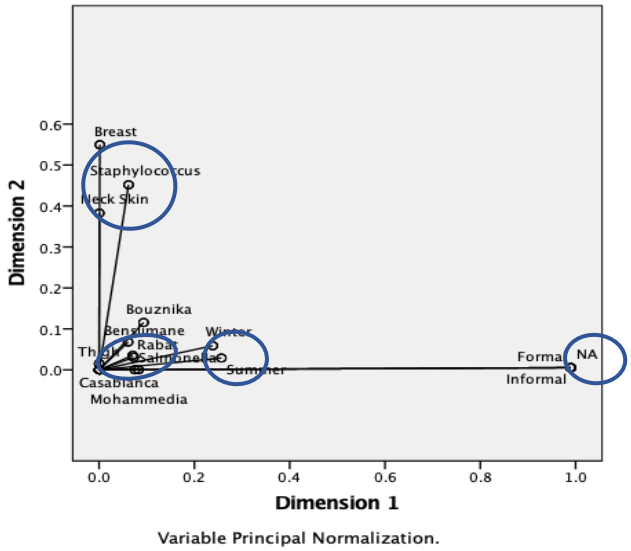


**Figure 3.** Variables differentiation analysis

**The cross-sectional study**

The present cross-sectional study included non-randomized comparison groups (exposed and unexposed) due to practical considerations in terms of time and data availability. The calculated sample size for an expected incidence in unexposed equaled 0.03, with a test power of 80% and a level of bilateral trust of 95%. To gain a risk ratio (RR) of 3, a total sample size of 486 samples was needed (243 per group, including exposed and unexposed).

**Comparison according to the sample type**

The *Salmonella* spp. and *S. aureus* loads in chicken meat were not the same regardless of the sampling type. There was strong evidence of an association between a specific sampling type and an overload of *Salmonella* spp. and *S. aureus* in chicken meat products. The RR estimate could be higher if sampling was on the neck skin and thigh than on the breast. For *Salmonella* spp., the RR estimate was about 3.5 (CI: 1.39-8.79, $p < 0.05$). The RR was significantly higher for *S. aureus*, with a RR of 6.66, compared to *Salmonella* spp. (CI: 2.96-14.9, ($p < 0.05$).

**Comparison according to the sector**

The risk of contamination of meat products with Salmonella spp. and S. aureus was significantly higher in the informal than in the formal sector. The RR estimate for *Salmonella* spp. and *S. aureus* was, respectively, 11.11 (CI: 7.86-14.35, $p < 0.05$) and 3.08 (CI: 1.72-5.52, $p < 0.05$).

**Comparison according to the size of the city**

The RR estimation was carried out only for the informal sector. It revealed a high risk of contamination by *S. aureus* in small-sized cities (Benslimane and Bouznika, RR = 1.77, CI: 1.10-2.83, $p < 0.05$). Exposure to small-sized cities determinant seems to be driving protection against *Salmonella* toxi-infection (RR < 1), but the RR estimate was not significant (RR = 0.66, CI: 0.30-1.43, $p > 0.05$).

**Comparison according to the seasonal pattern**

Regarding RR, meat handling and processing were subjected to contamination by *S. aureus* and *Salmonella* spp. in the summer period than in the winter with a RR of 1.46 for *S. aureus* (CI: 0.96-2.22, $p > 0.05$) and 1.10 for *Salmonella* spp. (CI: 0.61-1.98, $p > 0.05$). However, considering the p value, it was difficult to validate the significance of the association.

## DISCUSSION

The statistical analyses showed a significant difference between the prevalence of both investigated bacteria in the formal and informal sectors ($p < 0.05$). The prevalence of *S. aureus* and *Salmonella* spp. was higher in the informal sector compared to the formal sector, which might result from better control of the production chain and hygiene conditions in the formal sector. Taking into consideration that the informal sector is the main supplier of broiler chicken meat to the Moroccan market, it represents a higher risk of food contamination (FISA, 2022b) and remains a potential



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source of pathogens and a direct cause of enteric diseases worldwide, especially in developing countries (Adesiji et al., 2011; Akbar and Anal, 2013).

The results of this study revealed that 40/540 broiler chicken meat samples of both sectors (7.40%) were contaminated with *Salmonella*. This is in agreement with the previous studies, which found a prevalence of 7.30% and 6.67% of *Salmonella* spp. in broiler chicken meat purchased from poultry slaughterhouses in Thailand and Brazil, respectively (Chotinun et al., 2014; Panzenhagen et al., 2016). Moreover, Gu et al. (2020), Assèta et al. (2011), and Abba et al. (2017), respectively found the prevalence of 57% and 37% and 34.15% *Salmonella* spp. in broiler chicken meat samples from poultry slaughterhouses in China and open markets in Burkina Faso, and at points of sale in the markets of the city of N'Djaména, Tchad. In addition, the prevalence of *Salmonella enteritidis* in the present study was 67.5%. These findings are supported by a previous study that showed a prevalence of 55.7% of *Salmonella enteritidis* in turkey and broiler carcasses in Southern Brazil (Ruban et al., 2012).

The presence of *Salmonella* spp. in broiler chicken meat could be attributed to the lack of proper cold chains, inadequate power supply, and poor hygiene at retail outlets (Ruban et al., 2012). *Salmonella enteritidis* was also identified as the most common serotype in human cases; it was mainly found in broiler chicken meat and laying hens (Gu et al., 2020). Moreover, it was reported that *Salmonella* spp. contamination in broiler chicken meat decreases with the modernization of the slaughter process (Ruban et al., 2012).

This bacterium also colonizes a high percentage of broilers during fattening. The skin and meat of carcasses are frequently infected with the pathogen during slaughter and processing (FAO, 2022). several studies have reported *Salmonella* prevalence in broiler farms as 34.37%, 24%, 19.9%, in Algeria, Morocco, and Tunisia, respectively (Chaiba and Rhazi Filali, 2016; Djeffal et al., 2018; Oueslati et al., 2021). Therefore, it is important to consider farm contamination, which represents a critical stage in the development of *Salmonella*, both in terms of its impact on public health and the significant economic repercussions it can generate (Chaiba and Rhazi Filali, 2016).

Another study noted that 15.92% of broiler chicken meat samples from both sectors were contaminated with *S. aureus*. Similar rates were reported in Thailand and Morocco, with 18.18% and 16.66%, respectively (Akbar and Anal, 2013; Khallaf et al., 2014). A higher prevalence of *S. aureus* was reported in traditional slaughterhouses, compared to modern slaughterhouses (11.11% and 6.66%), which is in line with the results of Khallaf et al. (2014) at 27% and 8%, respectively. The results of the current study were indicative of the non-respect of good hygiene practices in traditional slaughterhouses, affecting the hygienic quality of broiler chicken meat.

The majority of the traditional slaughterhouses from which the samples were taken did not meet the minimal hygiene standards requirements, indicating the presence of *S. aureus*. In addition, the employers in these slaughterhouses did not undergo physical examinations, wore unclean and working uniforms, and worked in questionable hygienic conditions.

The use of modern slaughtering installations could considerably reduce the bacterial load in chicken meat, as was found in another study with a lower prevalence of *S. aureus* and absence of *Salmonella* spp. (Ruban et al., 2012). It was also found that there was a significant difference between the types of samples analyzed regarding *S. aureus*; a higher prevalence was found in the neck skin (59.3%), which could be explained by poor hygiene conditions and handling. In addition, a higher prevalence of *Salmonella* spp. was found in the thighs (57.5%), which can be due to their proximity to the point of evisceration and the maximum handling of the thigh region during dressing operations.

According to the results of MCA, the present study revealed positive samples (*Salmonella* spp. and *S. aureus*) when meat products were manipulated and processed in the informal sector, small cities, and during the summer season (Group I). Conversely, the negative samples (*Salmonella* spp. and *S. aureus*, group II) belonged to a context with best hygiene practices and efficient veterinary control (formal slaughterhouse factory, big-sized cities, winter period). To assess the effect of seasonality and size of the cities on the bacterial load in meat products, the study considered the primary measures, including refrigeration devices and rigorous good hygiene practices for the formal sector, which could considerably limit the bacterial load. Formal factories were not severely understaffed or suffered weaker links to the hygiene control process. Therefore, the season and city size factors were tested separately only for data from the informal sector. This compromised the sample size to 33% reduction and would limit the significant association, as mentioned above in the result section.

Regarding the *in-vitro* antibiotic sensitivity test results, several studies have focused on the antibiotic resistance of *S. aureus* and *Salmonella* spp. strains in poultry farms as well as in industrial or traditional poultry slaughterhouses. It was reported that high rates of *Salmonella* spp. resistance to tetracycline in chicken meat (Andoh et al., 2016; Abba et al., 2017). El Allaoui et al.(2017) found a high resistance level (79%) to tetracycline in Moroccan broiler turkey farms. On the other hand, Odoch et al. (2017) reported a relatively lower rate of tetracycline resistance (5.1%). The difference in the tetracycline resistance rates found could be explained by variability in the frequency and method of this molecule use.

In the current study, isolated *Salmonella* strains commonly showed resistance to ciprofloxacin and nalidixic acid and significant susceptibility to cefoxitin, gentamycin, and trimethoprim-sulfamethoxazole, similar to some other studies



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(Andoh et al., 2016; El Allaoui et al., 2017; Yang et al., 2010). Quinolones were part of the treatment of typhoidal salmonellosis. Although nalidixic acid has good activity *in vitro*, it was inefficient at the clinical practice level, probably due to its low bioavailability (Marchou and Meurisse, 1992), unlike fluoroquinolones, such as ciprofloxacin, having a higher oral bioavailability and have been the reference treatment for non-typhic salmonellosis for a decade (Marchou and Meurisse, 1992).

The wide use of antibiotics in veterinary and human medicine caused the emergence of *in vitro* resistance in certain serovars with failures to ciprofloxacin in some countries, such as the United Kingdom, India, Slovak Republic, and France (Piddock LJV, 1990; Weill et al., 2006). This could be related to the non-cautious use against other diseases, or illicit use of these molecules in food additives, or even acquired genes (Andoh et al., 2016). Moreover, *Salmonella* resistant to ciprofloxacin is usually resistant to several other antibiotics (Cui et al., 2008) and are associated with significant morbidity and mortality (El Allaoui et al., 2017). According to El Allaoui et al. (2017), the isolated *Salmonella* strains have a 27.50% resistance rate to kanamycin. However, high sensitivities were observed with cefoxitin (97.50%), trimethoprim-sulfamethoxazole (90%), and gentamycin (95%). This could be explained by the fact that aminoglycosides, especially gentamycin, have shown good activity against several human pathologies. Gentamycin is one of the most rarely recommended antibiotics in poultry farming because of its parenteral administration mode (Elared et al., 2001). According to the results of the present study, all *S. aureus* strains showed resistance against ampicillin, similar to same as a study conducted in Nigeria (Awogbemi et al., 2018). The high tetracycline resistance determined in previous studies was similar to that found in the present study (Kraushaar et al., 2017; Lika et al., 2021). Additionally, high antimicrobial resistance rates were demonstrated against kanamycin 74.41% in accordance with the results obtained in South Africa (Mkize et al., 2017). *Staphylococcus aureus* isolated from the chicken meat samples demonstrated moderate resistance to erythromycin and streptomycin with 54.65% and 51.16%, respectively, which was also reported in other studies (Mkize et al., 2017). However, 94.18% of *S. aureus* strains were susceptible to trimethoprim-sulfamethoxazole.

Based on the obtained results, it was observed that 97% of *Salmonella* spp. isolates were resistant to at least one antibiotic, while 30% were resistant to more than three antibiotics. All *S. aureus* isolates showed resistance to at least one antibiotic and a significant rate of 86% of resistance to more than three antibiotics. This high rate of multi-resistance to antibiotics is probably due to inappropriate treatment, either overtreatment or short treatment, or even inadequate treatment of these antibiotics. This situation can significantly impact the epidemiology of human salmonellosis, and thus considerably limit the choice of antibiotics for therapy (Elared et al., 2001). Therefore, regular and close monitoring and rationalization of poultry antibiotic use is recommended.

## CONCLUSION

Present results indicated that broiler chicken meat purchased from traditional poultry slaughterhouses of the study area (Ryachates) is mostly contaminated by *Salmonella* spp. and *S. aureus*. These Ryachates are mostly approached by Moroccan citizens, and directly threaten the consumers' health. Therefore, it is difficult to treat infected patients with antibiotic resistance. Therefore, to overcome this situation, a close collaboration of the staff in different sectors is required for attention to the hygiene standards and regulations in force. Therefore, future studies can be conducted to investigate a molecular study of the strains by sequencing and metagenomic analysis to determine the relationship between animal health and human health. Moreover, it is important to determine the minimum inhibitory concentrations (MIC) of the antibiotics used for virulence genes in each isolated bacterium.

## DECLARATIONS

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### Authors' contribution

Sabrine Nacer and Sophia Derqaoui collected samples used in this study. Sabrine Nacer and Fatima Zahra El Ftouhy performed the analysis in the laboratories. Sabrine Nacer and Mounir Khayli contributed data analysis. Sabrine



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Nacer wrote the original draft. Mustapha Lkhider and Saadia Nassik revised and edited the draft and generated the final version of the manuscript. All authors contributed to the article and approved the submitted version.

**Competing interests**

The authors declare that there is no conflict of interest.

**Data availability**

The authors declare that they have all the necessary data and are available where appropriate or requested by the editor.

**Ethical considerations**

Ethical considerations (including plagiarism, consent to publish, misconduct, fabrication and/or falsification of data, dual publication and/or submission, and redundancy) were checked by all authors.

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