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Original Article

Prevalence and genotypic characterization of *Salmonella* spp. from chicken meats marketed in the province of Skikda, Algeria

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Abstract

Here, we aim to determine the prevalence of *Salmonella* contamination of poultry meat from butcheries of the province of Skikda and to investigate antibiotic resistance. *Salmonella* spp. isolates were screened from 70 samples, including chicken breasts (n = 40 samples) and chicken thighs (n = 30 samples) collected from 14 butcheries. All suspected *Salmonella* colonies from selective media were confirmed by MALDI-TOF MS and serotyped. The susceptibility profile to 16 antibiotics was studied. According to the antibiotic susceptibility results, resistance genes were investigated by standard PCR targeting various genes such as *bla*$_{SHV}$, *bla*$_{TEM}$, *aac3*, *aac6-Ibcr*, *aad*, *qnrA* and *qnrB*. Of the 14 butcheries studied, samples from eight butcheries were contaminated with *Salmonella* (57.14%). 19 *Salmonella* strains were isolated, including five serotypes with a predominance of Kentucky serotype (n = 9), Enteridis (n = 3), followed by Heidelberg (n = 3), Virchow (n = 3), and Manhattan (n= 1). All isolates were resistant to Rifampicin (100%; n = 19), and to other antibiotics such as Ciprofloxacin (47.36%), Amoxicillin-clavulanic acid (47.36%; n = 9), Amoxicillin, (47.36%; n = 9), Ticarcillin-clavulanic acid (47.36%; n = 9), and Gentamycin (47.36%; n = 9). All isolates showing multidrug resistance (47.36%; n = 9) were positive by PCR to the *bla*$_{TEM-1}$ β-lactamase gene, from which 8 strains carried the aminoglycoside resistance *aad*7 gene. However, none was positive for the tested *bla*$_{SHV}$, *Aac3*, *Aac6-Ibcr*, *qnr*A, qnrB, *Arm*A and *Arm*B genes. Our findings show a worrying rate of *Salmonella* contamination of poultry meats.

Key words: Antibiotic resistance; butcheries; *Salmonella*; white meat, Algeria.

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Introduction

Salmonella is one of the most important causes of foodborne diseases worldwide. It is frequently associated with consumption of contaminated products such as poultry, eggs, meat, milk and seafood [1]. *S. enterica* infection leads to severe public health consequences and significant economic losses [2]. Algeria has seen a significant development of the poultry industry over the last decade and chicken meat is the most popular because of its relatively low price and easy digestibility [3,4]. According to the statistics of the Ministry of Agriculture, Algeria produces about 460,000 tons of white meat and 6 billion eggs annually [5]. In Algeria, the poultry meat contamination occurs during the transport of live birds, their housing, slaughter and marketing without compliance with basic hygiene criteria. This meat is generally implicated in human salmonellosis outbreaks causing acute gastroenteritis, especially in young and immuno-deficient patients [6]. Furthermore, poultry has been reported as a source of non-typhoidal *Salmonella* resistant to clinically relevant antibiotics with a higher incidence in middle-income countries [7]. The emergence and spread of antimicrobial-resistant *Salmonella* strains, particularly multi-drug resistant (MDR), is a major public health concern [7]. Genes conferring resistance to these antibiotics have been found on different plasmid types. The latter carry multiple antibiotic resistance genes that are transferable to other *Salmonella* strains and other bacterial species [7]. In this scope, the present study was undertaken to study the prevalence of *Salmonella* contamination in marketed poultry meat in Skikda province and to

characterize the antibiotic resistance mechanisms of the *Salmonella* isolates.

Methodology

Study locations

The present study was carried out from 14 butcheries, located in the province of Skikda (northeastern Algeria), over a period from December 2014 to February 2016. We have tried to cover the most accessible municipalities of the province. For technical reasons, including purchase of poultry meats, a total of 70 samples were collected. Samples consisted of three breasts and two thighs. All samples were transported to the laboratory into ice packs within a period not exceeding two hours to be treated on the same day or kept in the refrigerator overnight.

Data collection and analysis

Bacteriological analyses were performed according to the EN/ISO 6579-2002/Amd1:2007 protocol for *Salmonella* detection in food and animal feedstuffs [8]. Samples (25g) of meat and skin of breast and thigh were individually pre-enriched with 225 mL of buffered peptone water broth (PWB) (Fluka, Sigma Aldrich, St. Quentin Fallavier, France). All samples were incubated at 37°C for 18-20 hours. From each pre-enrichment solution, 1 mL and 0.1 mL were respectively transferred into 10 mL of enrichment Muller-Kauffmann tetrathionate / novobiocin broth (AES Chemunex Combourg, Bretagne, France) and 10 mL of Rappaport Vassiliadis broth (Merck Darmstadt, Land Hessen, Germany) and incubated at 37 °C and 42 °C for 24 hours, respectively. Both enriched samples were then streaked on XLD (Fluka analytical Steinheim, Buchs, Switzerland) and Hektoen agars (Pasteur Institute of Algeria) and incubated at 37 °C for 24 hours. Suspected colonies were first identified with the API 20E System (bioMérieux, Crappone, France), then confirmed with MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization Time OF light Mass Spectrometry) (Bruker Daltonics GmbH, Germany) [9]. The protein mass profiles were obtained using the Microflex LT MALDI-TOF mass spectrometer (Bruker Daltonics, Brême, Germany), with Flex Control software (Bruker Daltonics, Brême, Germany). The spectrum profiles obtained were visualized with Flex analysis v.3.3 software and exported to MALDI-Biotyper v.3.0 (Bruker Daltonics, Germany) for data processing (smoothing, baseline subtraction and spectra selection). The phyloproteomic analysis of *Salmonella* strains was assessed through construction and comparison of their reference spectra (main spectra) with the MALDI-Biotyper v.3.0 software (Bruker Daltonics, Germany). Cluster analysis was performed based on a pairwise comparison of specific main spectra (MSP: mean spectra projection dendrogram) of the different strains to generate a dendrogram of similarities among spectra profiles using the software default correlation function.

Confirmed *Salmonella* isolates were serotyped according to the Kauffmann-White-Le Minor's scheme [10]. Antibiotic susceptibility test was determined on Mueller-Hinton agar by standard disk diffusion procedure, as described by the European Committee on Antimicrobial Susceptibility Testing [11]. The *Salmonella* isolates were tested for amoxicillin (25µg), amoxicillin / clavulanic acid, ticarcillin / clavulanic acid, ceftriaxone, cefoxitin, cefotaxime, imipenem, ertapenem, aztreonam, gentamicin, amikacin, ciprofloxacin, colistin, rifampicin, trimethoprim / sulfamethoxazole and fosfomycin.

PCR Detection and sequencing of ESBL genes

Screening for resistance genes focused on a subset of isolates selected according to their resistance phenotype. The presence of the resistance genes in these isolates was determined by different PCR assays. Total nucleic acids were extracted using a BioRobot EZ1 Advanced XL instrument (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Detection of β-lactamase genes (including bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$), fluoroquinolones genes (*qnr*A, *qnr*B), aminoglycoside genes (AG)-modifying enzymes (*aac 3*and *aac(6')-Ib-cr*) was carried out by polymerase chain reaction (PCR) using specific primers: $bla_{\text{CTX-M-1 group}}$ [12], $bla_{\text{CTX-M-9 group}}$ [13], $bla_{\text{TEM group}}$ [14], bla_{SHV} [15], *qnr*A, *qnr*B [16], AME-encoding genes [*arm*A, *aad*, *aac(6)-Ib*] [17] and for MCR-1-encoding gene [18]. Positive PCRs were verified by electrophoresis using agarose gels containing SYBR safe (Invitrogen, Leek, the Netherlands), along with a DNA molecular weight marker (Benchtop pGEM®DNA Marker, Promega, Madison, Wisconsin, USA). Visualization of gels was carried out using the Benchtop pGEM® DNA Marker (Promega, Madison, Wisconsin, USA) under ultraviolet illumination. Positive PCR products were purified using the NucleoFast 96 PCR plate (Machery-Nagel EURL, RIORGES, France) and sequenced using the BigDye terminator chemistry on an ABI3730 automated sequencer (Applied Biosystems, Foster City, California, USA). The obtained sequences were blasted against the ARG-ANNOT database [19].

Results

Prevalence of poultry meat contamination by Salmonella

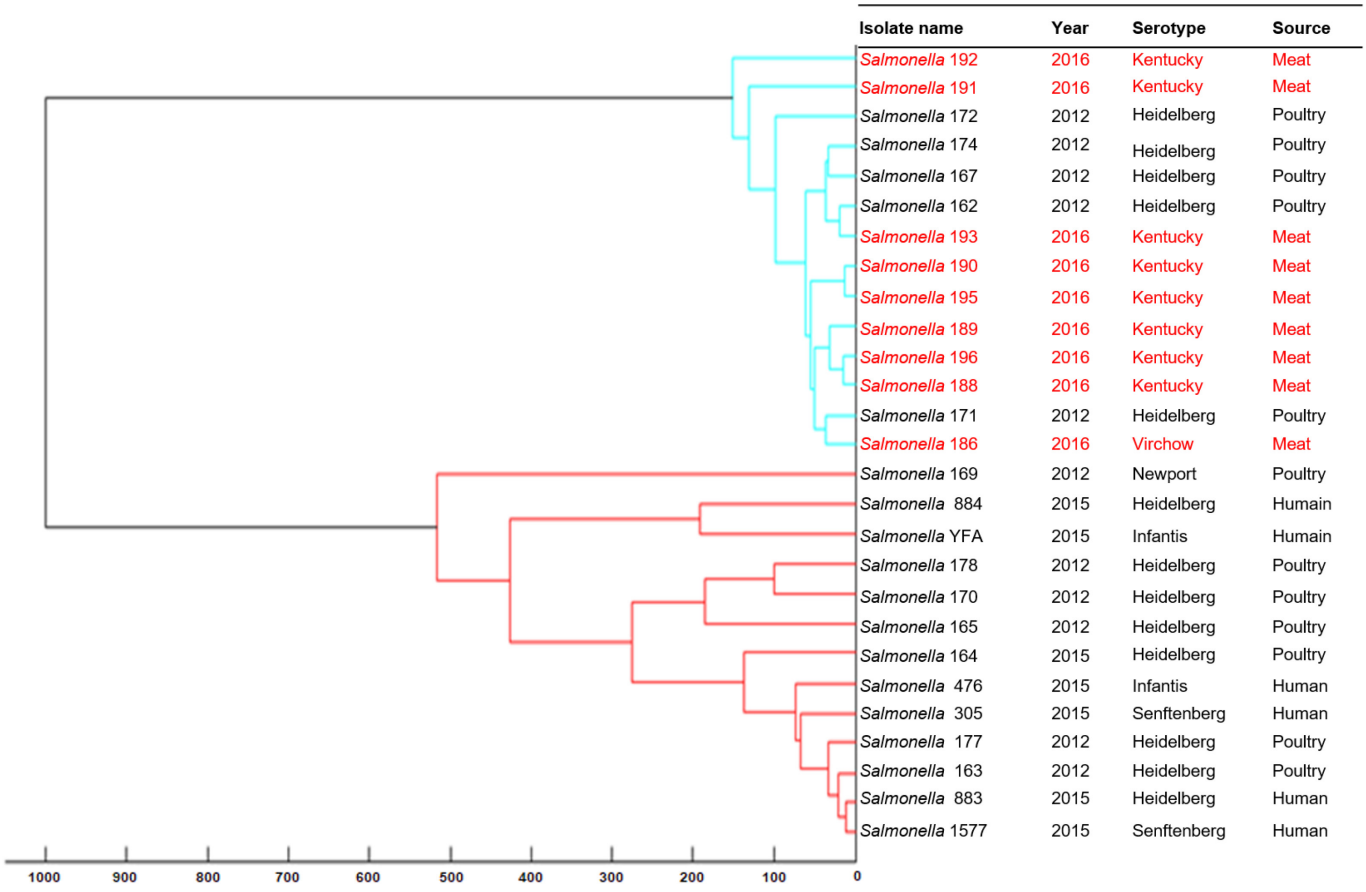
Of the 14 butcher shops studied, eight had poultry meat contaminated with *Salmonella*, resulting in a prevalence rate of 57.14%. The number of contaminated samples with *Salmonella* varied according to the nature of the sample: 10 breasts (n = 40), and 9 thighs (n = 30).

Distribution and antimicrobial resistance of Salmonella serotypes

Nineteen *Salmonella* strains were isolated from poultry meat samples and confirmed by MALDI-TOF MS and gave very good scores ranging from 2.00 to 3.00. The phyloproteomic analysis of the multidrug resistant *Salmonella* strains (n = 9) from poultry meats of the present study and isolates from human and poultry of our previous study [20] was assessed through the construction and comparison of their characteristic reference spectra (main spectra). As shown on Figure 1, we noted that all *Salmonella* strains from poultry meats and five poultry farms avian strains clustered together (A distance level of 150). Interestingly, we observe that *Salmonella* strains of the present study (in red) clustered together with strains of our previous study, suggesting the presence of a *Salmonella* clone which contaminate poultry meats and farm environment.

Five serotypes from nineteen *Salmonella* strains were identified with a predominance of the serotypes Kentucky (n = 9), Enteritidis (n = 3) followed by Heidelberg (n = 3), Virchow (n = 3) and Manhattan (n = 1). Butchery isolates were found to be resistant to Rifampicin (100%). Among 19 isolates, only nine isolates exhibited antibiotic resistance phenotype. We also noted resistance to other antibiotics (Table 1), such as Ciprofloxacin (n = 9, 47.36%), Amoxicillin-clavulanic acid (n = 9, 47.36%), Amoxicillin (n = 9, 7.36%), Ticarcillin-clavulanic acid (n = 9, 47.36%), and Gentamycin (n = 9, 47.36%). All tested isolates were susceptible to colistin. Nine strains carried bla_{TEM} gene while eight strains (10%) carried *aad* genes. Sequencing of bla_{TEM} and *aad* PCR products and Blast analysis of these sequences reveals the presence the β-lactamase bla_{TEM-1} and the aminoglycoside resistance *aadA7* gene. However, PCR search was negative for the

Figure 1. Dendrogram of resistant *Salmonella* strains isolated from chicken meat, farms, slaughterhouses and human. The multidrug resistant *Salmonella* isolates in the present study (colored in red) are compared with isolates of our previous study [20].



bla$_{SHV}$, *aac3*, *aac6-Ibcr*, *qnr*A and *qnr*B and *mcr*-1 genes.

Discussion

The results of the present study highlight the prevalence of contamination of poultry meat sold in some butcheries in the province of Skikda. The recorded prevalence rates (57.14%) are less with those reported in other similar studies 35, 5% in Mexico [21; 33% in Niger [22]; 22.6% in Egypt [4] and 5.92% in Saudi Arabia [23]. As previously reported, white meat had significantly more bacteria than other types of meat [24-27]. Poultry and cutting meats are often contaminated with the gastrointestinal flora, which could possibly be the cause of food borne pathogens [28].

Contamination from one commodity to another, usually through the hands of operators, utensils and work tops or cutting boards [29]. Studies in this area clearly show that without adequate precautions, the bacteria present on the surface of chicken carcasses can be disseminated in the kitchen after raw meat cutting operations [30,31]. We have noted a higher contamination of the breast meat (23.80%). This finding is in accordance with the results reported by Khalafalla *et al*. with *Salmonella* contamination of 20% for breast meat and 33.3% for thighs [4].

The results of the serotyping showed a very heterogeneous distribution of the serotypes. This distribution suggests that most *Salmonella* serotypes are primarily transmitted during breeding and slaughter.Two serotypes were identified in most of the butcheries studied namely, Kentucky (n = 9) and Enteritidis (n = 3). Khalafalla *et al*. also recorded the predominance of serotype Enteritidis in their study on butcheries in Egypt [4]. This serotype is also very common in animal production, especially in poultry farming [32]. The white meat isolates were predominantly resistant to rifampicin. These results agreed with Abd El-Tawab *et al*. who reported 100% of resistance to cephalexin and rifampicin in *Salmonella* isolated from chicken meats in Dubai [33]. We have noted resistance to certain antibiotics, such as ciprofloxacin; amoxicillin-clavulanic acid, amoxicillin; ticarcillin-clavulanic acid and gentamycin. Penicillin and tetracycline are commonly used in poultry feed as antimicrobial agents. The resistance to these antimicrobials has been previously demonstrated and related to poultry production units [34,35]. By comparing our results with those of a study carried out in the same region on farms and slaughterhouses [20], it appears that antibiotic resistance in poultry meat is less expressed, which reinforces the hypothesis that amplifying factors such as breeding conditions (drinking water, nature of the soil on the farm and in the slaughterhouse) are involved in *Salmonella* contamination. Shea suggested that prolonged antibiotic therapy is the major cause of antimicrobial resistance. Fluoroquinolones are among the most widely used antibiotics for treating human and animal salmonellosis because of their broad antimicrobial activity spectrum [36]. The most prevalent serovar

Table 1. Antimicrobial resistance and resistant genes profiles of MDR *Salmonella enterica* strains isolated from poultry meat.

Strain ID N°	Origin	Antimicrobial resistance pattern	Serotype	Resistance genes
179	Breast	RA	*Heidelberg*	/
180	Breast	RA	*Heidelberg*	/
181	Breast	RA	*Heidelberg*	/
182	Breast	RA	*Manhattan*	/
183	Breast	RA	*Enteritidis*	/
184	Breast	RA	*Enteritidis*	/
185	Thigh	RA	*Enteritidis*	/
186	Breast	AMX, AMC, TIM, CN, CIP, RA	*Virchow*	*bla*$_{TEM-1}$
187	Thigh	RA	*Kentucky*	/
188	Thigh	AMX, AMC, TIM, CN, CIP, RA	*Kentucky*	*bla*$_{TEM-1}$, *aadA7*
189	Breast	AMX, AMC, TIM, CN, CIP, RA	*Kentucky*	*bla*$_{TEM-1}$, *aadA7*
190	Breast	AMX, AMC, TIM, CN, CIP, RA	*Kentucky*	*bla*$_{TEM-1}$, *aadA7*
191	Thigh	AMX, AMC, TIM, CN, CIP, RA	*Kentucky*	*bla*$_{TEM-1}$, *aadA7*
192	Breast	AMX, AMC, TIM, CN, CIP, RA	*Kentucky*	*bla*$_{TEM-1}$, *aadA7*
193	Thigh	AMX, AMC, TIM, CN, CIP, RA	*Kentucky*	*bla*$_{TEM-1}$, *aadA7*
194	Thigh	RA	*Virchow*	/
195	Thigh	AMX, AMC, TIM, CN, CIP, RA	*Kentucky*	*bla*$_{TEM-1}$, *aadA7*
196	Thigh	AMX, AMC, TIM, CN, CIP, RA	*Virchow*	*bla*$_{TEM-1}$, *aadA7*
197	Thigh	RA	*Kentucky*	/

AMX: Amoxicillin; AMC: Amoxicillin/Clavulanic acid; CRO: Ceftriaxone; TIM: Ticarcillin/Clavulanic acid; RA: Rifampicin; CN: Gentamicin; CIP: Ciprofloxacin; RA: Rifampicin.

carrying resistance genes was *Salmonella* Kentucky. It has become the most commonly detected serovar in chickens, while *S.* Typhimurium remains the most common cause of human infections. The prevalence of multidrug resistance (MDR) *S.* Kentucky isolates from poultry is significant [37].

The present study demonstrates the presence of TEM genes. This finding is partly consistent with the results of previous studies, which confirmed the presence of β-lactamase encoding the bla_{TEM} gene conferring resistance to penicillins and first-generation cephalosporins [38,39]. ESBLs are mostly located on mobile genetic elements (plasmids or integrons) that can facilitate their mobility from a bacterial species to another by horizontal gene transfer [38]. We have reported the presence of *aad* genes that confer resistance to streptomycin, gentamicin and tobramycin. Aminoglycoside resistance in *Salmonella* is generally associated with the expression of aminoglycoside-modifying enzymes [40]. Our results are in accordance with those of Djeghout *et al*., who reported the presence of *aad*A7, *aad*A2 and *aad*A3 genes on most of streptomycin-resistant strains of *Salmonella* isolated from human and poultry in four Algerian cities [41]. Moawad *et al*. [38] and Sheng *et al.* [42] reported the presence of the *aad*A2 gene in isolates from retail meats in Egypt and Japan respectively [38,42]. Moreover, in Algeria, several studies have reported various contaminations of avian products by *Salmonella* spp. These contaminations may take place through the food chain, occupational exposure or direct contact with live animals and their environment in the broiler chicken industry [41,43,44,45].

Conclusions

The results of the present study demonstrate the existence of a worrying rate of *Salmonella* contamination in poultry meat that is sold in butcheries of the Skikda province. The potential implications of contaminated surfaces (slaughterhouses, kitchens and butcher shops) in the direct transmission of highly pathogenic micro-organisms such as *Salmonella* spp. to poultry meat are very frequent. The emergence of antimicrobial resistance of *S. enterica* isolates is a serious public concern in Algeria. Significantly, high rates of resistance have been detected to penicillin, cephalosporins, fluoroquinolones and aminoglycosides. Presence of genes encoding for antibiotic resistance was confirmed. In perspective, it would be interesting to carry out similar or more extensive studies on much larger samples in order to compare the results and evaluate these circulating clones with those of the study we previously performed on farms and slaughterhouses in the same region [20].

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Authors' contributions

SD has actively worked on the isolation of *Salmonella* strains, identification of strains by mass spectrometry and their characterization (antibiotic-susceptibility testing, molecular typing of genes, sequencing), data interpretation, drafting the paper and revising it. BM participated actively in drafting the paper and critically revising it. RE conceptualization, methodology, supervision. OB conceived and designed the study. BC contributed in part to the study design and data analysis (Serotyping and antibiotic-susceptibility testing of meat strains). J-MR conceived and designed the study and contributed to the revision of the article. SMD conceived and designed the study and actively in drafting the paper and critically revising it.

Ethical approval

This study was conducted according to ethical guidelines that were controlled and approved by the scientific council of the Institute of Veterinary Sciences (Mentouri Brothers University, Constantine - 1, Algeria) and complied with the guidelines for animal care and use in research and teaching. It is worth noting that no live birds were used in this study.

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