



Detection and Antimicrobial Susceptibility of *Salmonella* spp. Isolated From Commercial Eggs in Tiaret Province, Algeria

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ABSTRACT

Salmonellosis is a significant public health problem worldwide. The current study aimed to investigate the presence of *Salmonella* spp. in commercial eggs of Tiaret province, Algeria, and evaluate the susceptibility of isolated strains to different antimicrobial agents. A total of 180 commercial eggs collected from various retail outlets (groceries, butchers, wholesalers, street vendors) were analyzed by conventional methods, and 13 *Salmonella* spp. isolates were tested on a panel of 7 antimicrobial agents using the disc diffusion method. Of 180 chicken egg content samples examined, the findings indicated that 13 (7.22%) were positive for *Salmonella* spp. Regarding the collection site, 2 (1.11%), 4 (2.22%), and 7 (3.88%) of *Salmonella* spp. isolates were detected from butchers, wholesalers, and street vendors, respectively. Most antibiotic discs have demonstrated widespread resistance with an incidence rate of 100%, including amoxicillin + clavulanic acid, ampicillin, nalidixic acid, and erythromycin. However, colistin sulfate, gentamycin, and tetracycline were more effective against *Salmonella* isolates. It can be concluded that the highest detection rate of *Salmonella* spp. was observed for street vendors, and the highest resistance was recorded for commonly used antibiotics in poultry production.

Keywords: Antimicrobial, Chickens, Commercial eggs, *Salmonella*, Tiaret

INTRODUCTION

Salmonellosis is considered one of the most common foodborne diseases. The World Health Organization (WHO) reported 94 million cases of non-typhoid *Salmonella* gastroenteritis worldwide, with annual deaths reaching 500,000 in 2018 (WHO, 2018). Furthermore, the incidence and severity of cases of salmonellosis have increased significantly in both developed and developing countries (WHO, 2014). In Algeria, over 5000 human cases are reported yearly, but the real number of infections is likely higher (Fourar, 2019).

Poultry products are the most incriminated in most traceable foodborne diseases caused by *Salmonella* (Thorns, 2000). Chicken eggs, in particular, are considered the most potent food vectors of this bacterium for humans (Finstad et al., 2012). The contamination of eggshells and egg contents can occur during egg formation in the reproductive tract of laying hens or during processing and distribution due to poor hygienic practices (Moosavye et al., 2015).

Salmonella bacterium is a Gram-negative rod genus belonging to the *Enterobacteriaceae* family. Within two main species *Enterica* and *Bongori*, over 2500 serotypes or serovars have been identified to date. *Enterica* species with serovars *Enteritidis* and *Typhimurium* are considered to be the most common foodborne species that cause human salmonellosis (Bahness et al., 2015).

Over the past decade, antimicrobial resistance has become a severe public health problem worldwide (Ali and Mohamed, 2020; Mirzaei et al., 2022). Several researchers have reported the emergence of multidrug-resistant bacteria isolated from poultry products (Almashhadany, 2019; Xie et al., 2019; Merati et al., 2020). Antimicrobial resistance is a natural consequence of adapting infectious agents to exposure to antimicrobials in medicine and food animals. Therefore, an increase in the resistance, particularly of *Salmonella*, to commonly used antimicrobials has been noted in both public health and veterinary sectors, and multidrug-resistant phenotypes have been increasingly described among *Salmonella* species worldwide (Castro-Vargas et al., 2020). Monitoring antibiotic resistance among *Salmonella* isolates and controlling its health risk is essential.

Due to the lack of updated and accurate data in Tiaret Province, Algeria, about the antimicrobial resistance pattern of *Salmonella* spp. associated with commercial eggs, the present study investigated the presence of *Salmonella* spp. in the commercial eggs in Tiaret Province, Algeria, and evaluated the susceptibility of isolated strains to different antimicrobial agents.

MATERIALS AND METHODS

Ethical approval

This research was performed following the Veterinary Sciences Institute, University of Tiaret, Algeria guidelines.

Study area

The study was performed in Tiaret province, in Western Algeria. Geographically, the study site is located at a latitude of 35° 22' 15.71" N and a longitude of 1° 19' 1.16" E, and at an elevation average of 978 m above sea level.

Egg samples collection

A total of 180 unwashed chicken eggs were collected at random from different retail outlets (groceries [45], butchers [45], wholesalers [45], and street vendors [45]), located in Tiaret Province, under sterile hygienic conditions from January to May 2021. The samples were aseptically transported in an ice box to the hygiene and animal pathology laboratory, Tiaret, Algeria, for immediate bacteriological analysis.

Culture of *Salmonella*

Isolation procedures for *Salmonella* were carried out according to the recommendations of the International Organization for Standardization (ISO 6579, 2002). To collect the egg content, the surface of each chicken egg was disinfected with 75% alcohol, the shell was cracked with a sterile knife and carefully removed, each egg's content was mixed thoroughly and 25 g of the mixed egg content was inoculated into 225 ml of peptone broth and incubated at 37°C for 24 h. After the overnight incubation, 1 ml of the pre-enrichments broth was transferred aseptically into a tube containing 9 ml of Rappaport-Vassiliadis broth and incubated at 37°C overnight. Following incubation, a loopful of the broth was streaked onto one plate of xylose lysine desoxycholate agar (XLD; Biokar, France) and another plate of *Salmonella-Shigella* agar (SS; Biokar, France), and incubated at 37°C for 24 hr. Presumptive colonies for *Salmonella* isolates were then transferred onto nutrient agar (Oxoid, UK) and incubated aerobically at 37°C overnight (ISO, 2002).

Biochemical test

Salmonella suspected isolates were inoculated into triple sugar iron agar (TSI) slopes (Sigma-Aldrich, US) and incubated at 37°C for 24 hr. Typical *Salmonella* phenotypes were further confirmed with Analytic Profile Index (API) 20 E test strips (BioMerieux, France; Yang et al., 2015). All isolated strains were stored in peptone-glycerol solutions at -20°C for subsequent analysis.

Antibiotics susceptibility testing

The susceptibility of *Salmonella* isolates to antibiotics was examined by using the agar disc diffusion method on Mueller–Hinton Agar (MH; Biokar, France) according to the Standardization of Susceptibility to the National Scale Human and Veterinary (SSNSHV, 2011) guidelines. The following antibiotics manufactured for the analytical purpose were tested, amoxicillin + clavulanic acid (AMC: 30 µg; CYPRESS DIAGNOSTICS, Belgium), ampicillin (AM: 10 µg; CYPRESS DIAGNOSTICS, Belgium), tetracycline (TE: 30 µg; CYPRESS DIAGNOSTICS, Belgium), gentamycin (CN: 10 µg; Liofilchem, Italy), nalidixic acid (NA:30 µg; Liofilchem, Italy), erythromycin (E: 15 µg; Liofilchem, Italy), and colistin sulfate (CS:10 µg; Liofilchem, Italy).

Three to four colonies were suspended in 10 ml of 0.9% NaCl from a pure culture of each *Salmonella* isolate. The suspension was adjusted to match the turbidity standard of 0.5 McFarland units. Approximately 1×10^8 colony-forming units were streaked on MH agar plates using a sterile cotton swab, and the antibiotic discs were applied at the indicated doses. After aerobic incubation at 35°C for 20 h, the interpretation of the zones of inhibition was performed, and results were expressed as sensitive (S), intermediate (I), and resistant (R) according to the recommendation of SSNSHV (2011). An isolate was multidrug-resistant if it was resistant to at least one agent in three or more antimicrobial classes (Zhang et al., 2018).

Statistical analysis

Descriptive statistics were performed to compare proportions. The data were analyzed using Microsoft Excel 2016 (USA).

RESULTS

Bacteriological and biochemical examination

Out of 180 chicken egg samples examined, 13 (7.22%) were found positive for *Salmonella* spp. Regarding the collection site, 2 (1.11%), 4 (2.22%), and 7 (3.88%) of *Salmonella* spp. isolates were identified from butchers, wholesalers, and street vendors, respectively (Table 1). The isolates produced typical red-colored colonies with black

centers on the XLD agar medium. Colorless colonies with black centers were also produced on SS agar medium. Biochemical characterization performed on API 20 E revealed that all the isolates were positive for lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide, glucose, mannose, sorbitol, rhamnose, melibiose, and arabinose. In contrast, o-nitrophenyl-b-D-galactopyranoside, arginine dihydrolase, citrate, urease, Tryptophan deaminase, Indole, Voges-Proskauer test, gelatinase, inositol, sucrose, and amygdalin tests were negatives.

Antibiotic resistance of *Salmonella* spp. isolates

Salmonella isolates were tested against seven antibiotics. The results of susceptibility testing are illustrated in Table 2. All the isolates were resistant to amoxicillin + clavulanic acid, ampicillin, nalidixic acid, and erythromycin and immediately resistant to tetracycline. However, colistin sulfate and gentamycin were more effective against *Salmonella* isolates with 100% sensitivity. Regarding the multidrug-resistance results, all the isolates were resistant to three antimicrobial classes (Macrolide, Beta-lactam, and Quinolone); this means that 100% of the isolates were multidrug-resistant.

Table 1. Percentage of detected *Salmonella* spp. in different outlets isolated from commercial eggs in Tiaret, Algeria

Collection site	Number of examined samples	Number of Positive (%)
Groceries	45	00
Butchers	45	2 (1.11)
Wholesalers	45	4 (2.22)
Street vendors	45	7 (3.88)
Total	180	13 (7.22)

Table 2. Antimicrobial susceptibility results for 13 *Salmonella* spp. isolated from commercial eggs in Tiaret, Algeria

Antimicrobial agent	Disc concentration (µg)	<i>Salmonella</i> spp. isolates (n=13)			Percentage of resistance
		S	I	R	
Ampicillin	5	0	0	13	100
Amoxicillin + acide Clavulanique	10	0	0	13	100
Tetracycline	30	0	13	0	0
Gentamycin	10	13	0	0	0
Nalidixic acid	30	0	0	13	100
Erythromycin	15	0	0	13	100
Colistin sulfate	10	13	0	0	0

S: Sensitive, I: Intermediate, R: Resistant.

DISCUSSION

Salmonella has been the primary cause of the foodborne salmonellosis pandemic in humans over the last 20 years, during which egg products were the most often identified vehicle of the infection (Moosavy et al., 2015; Long et al., 2017). Over the past decades, the improper and uncontrolled use of antibiotics has allowed the appearance of strains of *Salmonella* with multiple drug resistance in many countries (Ben Salem et al., 2012; Nacer et al., 2022; Samy et al., 2022). For that reason, the antimicrobial resistance of *Salmonella* has become a matter of concern worldwide due to the significant threat that can present to public health (Wu and Hulme, 2021).

Considering the overall number of eggs collected, the results of the present study demonstrated that, of the 180 samples analyzed, 13 isolates of *Salmonella* spp. were detected at the rate of 7.22%. This partially agrees with the results shown by Hossain et al. (2019), who reported that the overall prevalence of *Salmonella* in the Naogaon district of Bangladesh was recorded as 7.78%, whereas 5.56% was on eggshell surfaces and 2.22% was on egg contents. In addition, Xie et al. (2019) found that 7.6% and 3.2% of farm and market egg samples were contaminated with *Salmonella* in Guangdong, China. In contrast, to the present findings, Zubair et al. (2017) reported that out of the 350 eggs analyzed, 17 (4.85%) samples of eggshells were found contaminated with *Salmonella* spp. and none of the egg content samples were contaminated with this bacterium. The same results were reported by Mansour et al. (2015) who recorded a total absence of *Salmonella* in the egg content samples. These observed variations can be explained by the different approaches used for the choice of samples (eggshell or egg content), and the methods used to isolate and identify the microorganism.

Regarding the detection rate of the bacterium with retail outlets, the street vendors presented the highest rate of *salmonella* contamination in relation to other outlets at 3.88%, followed by wholesalers and butchers at 2.22% and 1.11%, respectively. However, a complete absence of bacteria was noticed in grocery stores. It was suggested that street vendors' poor storage and marketing conditions may be the cause of the high level of egg contamination. Indeed, the egg's duration and storage temperature may have a disproportionate impact on subsequent contamination (Gantois et al., 2009).

Antimicrobial susceptibility results of *Salmonella* spp. isolated from table eggs marketed in the Tiaret area revealed that all strains (100%) were resistant to amoxicillin + clavulanic acid, ampicillin, nalidixic acid, and erythromycin. Several studies have reported different resistance rates; Islam et al. (2018) recorded that 94.44% and 77.78% of the *Salmonella* strains isolated from table eggs were resistant to amoxicillin and ampicillin, respectively. Likewise, Hossain et al. (2019) demonstrated resistance rates of 92.86% and 71.42% for amoxicillin and ampicillin, respectively. Unlike the present results, Xie et al. (2019) reported in a study carried out in China lower resistance rates of 12.96%, 37.04%, and 59.26% against nalidixic acid, amoxicillin, and ampicillin, respectively. The high rate of resistance found in this study can be related to the improper and uncontrolled use of these drugs in treating and controlling respiratory and digestive infections in poultry.

However, the results of the current study showed that all isolates were susceptible to gentamycin, tetracycline, and colistin sulfate. Several studies have reported different resistance rates to these antibiotics; Islam et al. (2018) recorded a resistance rate of 22.22% for gentamycin. In addition, Xie et al. (2019) reported low resistance to gentamycin with a rate of 18.52%, and higher resistance to tetracycline, with a rate of 42.59%. In contrast, the Zubair et al. (2017) group isolated 17 *Salmonella* strains from 350 analyzed table eggs and showed that all isolated strains were susceptible to gentamycin and colistin sulfate. These differences could be explained by the fact that each country has applied various policies and interventions to control the use of antibiotics in poultry production.

CONCLUSION

In conclusion, the current study's findings showed, firstly, that *Salmonella* spp. is detected in table eggs marketed in the area of Tiaret, and the highest detection rate was observed for street vendors. Therefore, significant attention should be paid to guaranteeing good quality eggs to the consumer, as *Salmonella* is considered one of the most important causes of human foodborne illness. Secondly, all *Salmonella* spp. isolates were resistant to amoxicillin + clavulanic acid, ampicillin, and nalidixic acid, while they were all susceptible to gentamycin, tetracycline, and colistin sulfate. The current study's authors suggest that the highest rate of resistance was observed for commonly used antibiotics compared to those used in specific poultry production cases. The overuse and misuse of antibiotics should be avoided in layer farms, as they are considered the main factors in the development of antibiotic resistance.

DECLARATIONS

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Authors' contributions

Rachid Merati contributed to the conception, design, data collection, analysis, and interpretation. Abdellatif Boudra contributed to the editing and writing the final draft of the manuscript. All authors approved the analyzed data and the last revised article.

Competing interests

The authors confirm that the data presented do not represent any conflict of interest.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and submission, and redundancy) have been checked by all the authors.

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