Antibiotic Susceptibility of *Salmonella* spp. Isolated from Chicken Feces

Kozhin M. A. Ahmed, Banaz M. Najmadin, Ayad H. Hasan and Bayan K. Jalal

Department of Medical Microbiology, Koya University, Daniel Mitterrand Boulevard, Koya KOY45 AB64, Kurdistan Region - Iraq

***Abstract*—*Chicken* feces sample was collected from 10 different farms around Koya city between November 25 and December 30, 2017. All samples were cultured and diagnosed by conventional methods; then antibiotic susceptibility test was carried out by the agar disc diffusion method. The fecal samples that contain *Salmonella* spp. were determined and examined against nalidixic acid (NAL) (30 µg), chloramphenicol (10 µg), azithromycin (AZM) (15 µg), and amoxicillin (AMX) (25 µg). Resistance in fecal *Salmonella* spp. to NAL, CHL, and AZM was 100%, 91.6%, and 83.3%, respectively. AMX resistance was observed in 50% of the *Salmonella* spp., and AMX sensitive was observed in 33.3% of the isolates. The remaining isolates showed 16.7% intermediate resistance to AMX. The results of this study strongly indicate that AMX can be used to inhibit or eliminate any source of foodborne *Salmonella* spp. infection.**

Index Terms*—Antibiotic resistant in chickens, Antibiotic susceptibility test, Poultry, *Salmonella.

I. Introduction

Chicken meat is a good source of protein that can be easily reached by most people, due to its inexpensive price and easy availability [1]. Close attention to chicken meat production is paid depending on the fact that many different genus of bacteria can live on the skin, feathers or in the alimentary tract of chickens. During the process of meat production, most if not all of these bacteria are eliminated. However, there is a possibility of contamination with pathogenic bacteria at any stage of the production process, starting from the slaughterhouse condition, feather plucking, disemboweling, cleaning to storage room [2,3]. Using an antibiotic by chicken farms as a prophylactic contributed to increasing the level of multidrug-resistant bacteria equally in human and animals [4]. These resistant bacteria can spread through the food chain and transfer its resistant genes to other human pathogens that cannot be successfully eradicate using the available antibiotics [5].

The most frequent bacteria that infect chickens and contaminate poultry products are *Salmonella* and *Campylobacter* [6]. In human *Salmonella* is one of the most common causes of bacterial gastroenteritis, which have been associated with many different foodborne infections, including poultry products [7,8]. This infection called salmonellosis that can be gained by eating undercooked contaminated poultry meat [9].

An emerging of *Salmonella* spp. isolates with antimicrobial resistance in humans and other poultry is a public health problem [10]. Obtaining data by some researches using pulsed-field gel electrophoresis supported the theory that chicken can be a source of multidrug-resistant *Salmonella* spp. in humans [11,12].

This bacterial resistance could be by one of the following mechanisms; modifying or destroying of the antimicrobial agent; pumping the antimicrobial agent out from the cell by efflux pumps; modifying the antibiotic target; and decrease in cell membrane permeability [13].

In addition, bacteria can develop resistance mechanism by stimulate a mutation in the gene, locations of target proteins or acquiring mobile genetic elements carrying resistance genes such as plasmid, integrons, and transposons [14].

This study aimed to isolate *Salmonella* spp. from chicken's feces and investigate their antibiotic-resistant properties against a different group of antimicrobial agents.

II. Materials and Methods

A. Fecal Sample Collection

From November 25 to December 30, 2017, a total of 30 fresh fecal samples were collected from 10 different chicken farms in Kurdistan, three samples from each farm. The samples were transferred to bacteriology laboratory on the day of collection to be diluted 1/10 in 0.9% NaCl containing 20% (v/v) glycerol and stored at −20°C until cultured and identified [15]. The chickens were aged between 14 and 50 days.

B. Specimen Culturing

Briefly, the samples were thawed to make 10–2 and 10–4 dilutions in 0.9% NaCl and then inoculated on *Salmonella*-Shigella agar plates (SS agar). All inoculated plates were incubated at 30°C for 24 h [16].

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Corresponding author's e-mail: ayad.hasan@koyauniversity.org

C. Gram Stain and Bacterial Identification

The isolated bacteria were subjected to standard Gram stain procedure, then observed under the oil immersion objective lens (×100). Isolated bacteria were identified using standard methods such as cultural characteristic and urease test [17].

D. Antibiotic Sensitivity Test

Disc diffusion method was used to carry out *Salmonella*'s resistance against nalidixic acid (NAL 30 µg), chloramphenicol (CHL 10 µg), azithromycin (AZM 15 µg), and amoxicillin (AMX 25 µg). The antibiotics were selected from deferent family groups based on their use in poultry on veterinary prescription [18]. Mueller-Hinton agar was used to conduct antibiotic sensitivity test. After suspending a loopful of isolated *Salmonella* in nutrient broth a 100 µl was spread over Mueller-Hinton plate by L-shape. Then, four different antibiotic disks were placed on the plate and incubated at 37°C for 12–24 h. After the incubation period, the diameter of inhibition zone was measured for each antibiotic by a ruler and compared with standard tables published periodically by clinical and Laboratory Standard Institute [18] based on that the bacterial isolates were classified as sensitive, intermediate, or resistant to the certain antibiotic.

III. Results

A. Bacterial Isolation and Identification

Twelve samples out of 30 were able to grow on SS agar. The colorless with black centered colonies were picked up for more investigation (Fig. 1 and Panel A). The black center forms as a result of hydrogen sulfide gas production by *Salmonella* spp. The selected colonies were inoculated on nutrient agar slant with 5% glucose. After 24 h of incubation at 37°C, all slants were stored in the refrigerator at 4°C. A loopful from each slant was subjected to Gram stain, which appeared as Gram-negative rod-shaped bacteria after exanimation under a light microscope at ×1000 (Fig. 1 and Panel B). The surface of individual urease agar slant was streaked with a single purified bacterial stock and incubated at 37°C for 24 h to make sure that our purified strains did not contaminate with Proteus spp. The examined bacterial samples could not change the medium color, which accounts as a negative result and confirms the purity of our isolates as *Salmonella* spp. (Fig. 1 and Panel C).

B. Antibiotic Sensitivity Test

All *Salmonella* spp. were resistant to NAL. The second antibiotic that recorded the highest incidence of resistance was CHL as 11 of 12 isolates (91.7%) showed resistance, and the remaining isolate being intermediately susceptible to that antibiotic. 10 of 12 isolates of *Salmonella* spp. (83.3%) showed resistant to AZM. AMX was the most effective antibiotic against *Salmonella* spp. 50% of the isolated bacteria were sensitive to this antibiotic (Table 1).

Susceptibility of *Salmonella* spp. Isolates Against Different Antibacterial Agents

In general, the resistance percentages of *Salmonella* spp. against AMX, AZM, CHL, and NAL were 50%, 83.3%, 91.7%, and 100%, respectively (Fig. 2).

IV. Discussion

Salmonella spp. are the common causative agent of a worldwide disease called salmonellosis, which can transfer to human through consumption of contaminated poultry products such as eggs and meat [9]. To cut the number of *Salmonella* infection, we need to eradicate them from the original source of infection. These can be done after understanding their antibiotic resistance

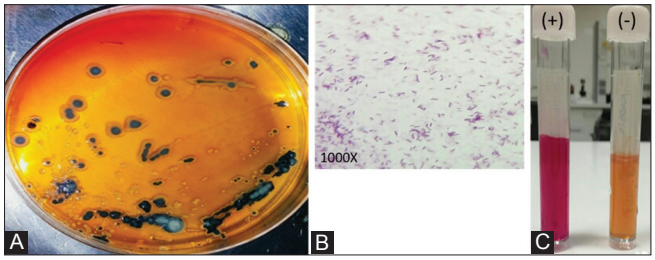

Fig. 1. Cultural characteristic, microscopic examination and urease test for selected faecal samples. Panel A shows a cultural characteristic of grown bacteria on *Salmonella*-Shigella agar. *Salmonella* spp. is non-lactose fermenters, which appear as a colorless colonies with black center that illustrates production of hydrogen sulfide. Panel B represents Gram stain examination of bacterial pure culture, which revealed a Gram-negative bacilli that refer to *Salmonella* spp. Panel C illustrates bacterial pure culture urease test. All purified samples manifested negative result without any change in medium color. The plus sign between brackets represents positive result, and the minus sign represents the negative result.

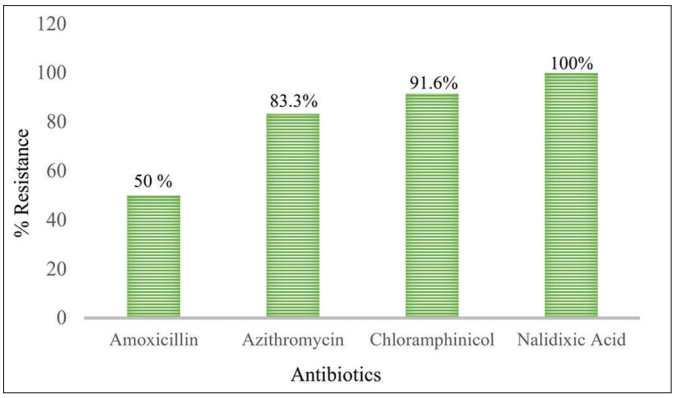

Fig. 2. Antibacterial resistance percentages of *Salmonella* spp. against nalidixic acid, chloramphenicol, azithromycin, and amoxicillin.

TABLE 1 Susceptibility of *Salmonella* spp. Isolates Against Different Antibacterial Agents

Susceptibility to	Percentage of Antibiotic Susceptibility Against			
	NAL N (%)	CHL N (%)	AZM N (%)	AMX N (%)
Resistance	12 (100)	11 (91.6)	10 (83.3)	6 (50)
Sensitive	0 (0)	0 (0)	2 (16.7)	4 (33.3)
Intermediate	0 (0)	1 (8.33)	0 (0)	2 (16.7)

NAL: Nalidixic acid, CHL: Chloramphenicol, AZM: Azithromycin, AMX: Amoxicillin

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pattern and mechanism. In this study, the susceptibility of isolated *Salmonella* spp. was tested against NAL (30 µg). Then, the nalidixic acid resistant isolates were tested by antimicrobial disc susceptibility method against CHL (10 µg), AZM (15 µg), and AMX (25 µg) based on CLSI guidelines [18].

Our results revealed that the isolated *Salmonella* spp. have a high level of resistance against the different verity of antibiotics. NAL resistance was observed in 100% (12/12) of the isolates. Resistance to NAL was stated as a result of mutation in the amino acid codon 87 of the *gyrA* gene, which substitutes of Asn in place of Asp [19,20].

CHL resistance was observed in 91.6% (11/12) of the *Salmonella* spp. isolates, and intermediate resistance was observed in 8.4% (1/12) of the isolates. There are two possible resistances mechanism against CHL in *Salmonella* spp. The first mechanism can be expressed by plasmid-located enzymes called CHL acetyltransferases or non-enzymatic CHL resistance gene *cm1A*. The second mechanism can be through efflux pump that pushing the antibiotic outside the cell [21].

Ten of 12 *Salmonella* spp. isolates (83.3%) were resistant to AZM, and the remaining isolates 16.7% (2/12) were sensitive to it. Resistance against AZM in *Salmonella* spp. include a mutation in *rlpD* or *rlpV*, presence of special genes such as *mphA*, *mphB*, *ermB,* or acquired of an efflux pump [22,23].

50% (6/12), 33.3% (4/12), and 16.7% (2/12) of the bacterial isolates were resistant, sensitive, and intermediate susceptible to AMX, respectively. β-lactamase is the enzyme that responsible for AMX resistance phenomenon, which is hydrolyzing the active compound in penicillin group antibiotics that called β-lactam ring and produce beta-amino acids with no antibacterial activity. In *Salmonella* spp. the encoding genes for β-lactamase are found on a plasmid [24].

The obtained results of antibiotic susceptibility test in this study showed that the AMX is the most potent antibacterial agent against *Salmonella* spp. isolated from chickens feces. Hence, AMX can be added to the chicken's food at a minimum inhibition concentration to weaken any existence of *Salmonella* in the food to be later killed by chickens own immune system. However, there are many ways can be taken to prevent *Salmonella* spp. from spreading. Of these, take extra caution during food preparation for infant, older people, and immunocompromised people. Cooking and storing food properly are another way to avoid any possible contamination that can happen by *Salmonella* spp.

V. Conclusion

This work highlighted that *Salmonella* spp., which isolated from chechen's feces showed a high level of resistance against NAL, CHL, and AZM. However, AMX showed effectiveness against 50% of the samples. This work could be extended by testing a wider range of antibiotics, to identify the most proper antibiotic to eradicate *Salmonella* spp.

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