Isolation and Antibiotic Resistance Profile of *Streptococcus Spp* from Commercial Poultry Carcasses in Ardabil Province, Northwest of Iran

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Abstract

Farmers use antibiotics for prophylaxis and therapy to maintain production. However, widespread use of antibiotics has increased antibiotic resistance. The present study was carried out to isolate and identify *Streptococcus spp* involved in commercial poultry carcasses and also to examine isolated bacteria for antibiotic resistance profile. Samples were taken from heart blood and liver's visceral surface of 125 carcasses obtained from 30 commercial flocks (including 27 broiler flocks, 1 laying flocks and 2 breeder flocks) located in different parts of Ardabil province, northwest of Iran. *Streptococcus spp* isolates were identified based on microbiological standard methods. The antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method. Out of 125 collected carcasses, ninety-eight (98) bacteria isolates were identified. Among them, 6 (6.13 %) of isolates were confirmed to be *Streptococcus spp*. The strains exhibited high percentages of resistance to Ampicillin (100 %) and Doxycycline (100 %) and low percentages of resistance to Clindamycin (16.7%) and Cefatoxaime (0.0 %). Neomycin, Enrofloxacin, Erythromycin, Gentamycin, Ciprofloxacin and Chloramphenicol were resistant with 83.4 %, 83.4 %, 66.7 %, 50.0 %, 33.4 % and 33.4 %. The present study showed that poultry products, especially infectious lesions of liver and heart of poultry carcasses can be an important source of contamination and dissemination of drug-resistant *Streptococcus spp*. Therefore, performing antimicrobial sensitivity test is necessary prior to prescribing any antibiotic in commercial poultry.

Key Words: *Antibiotic resistance, Streptococcus spp, Poultry carcasses, Ardabil province*

Introduction

Broilers have a high productivity, but their level of immunity against the disease is relatively low. In order to improve the immunity of chicken, farmers usually administering vitamins, vaccinations and antibiotics. Antibiotics are often used in poultry as a prevention of disease transmission and as a growth promoter. Antibiotics are also used for the treatment [1]

In spite of its beneficial to human and animal health, as well as increased productivity over the past few years, abusive use of antibiotics has led to resistance to pathogenic and commensal bacteria. [2, 3]. Over the past 50–60 years, bacterial resistance has spread [3, 4]. It is feared that resistant bacteria in chicken can infect humans, thereby reducing the effectiveness of treatment and caused death [1, 2, 3, 4, 5, 6].

Streptococcus spp. is a normal microflora in the mucosa digestive tract of broiler chickens [3]. Cases of *Streptococcus spp*. in chicken Broilers have been widely reported, but rarely reported as outbreaks. This matter occured due to infection with *Streptococcus spp*. generally subclinical and secondary [2]. Some diseases that can be caused this Gram-positive bacteria which include pneumonia, septicemia, cellullitis,
osteomyelitis, endocarditis and meningitis with a mortality rate of up to 50% [3].

The information about antibiotic resistance from this bacteria in commercial poultry of Iran is still limited.This study aligns with the overarching objectives of zoonotic disease research, emphasizing the need to monitor antimicrobial resistance in food-producing animals to assess potential spillover events. So, The aims of this study is to isolate Streptococcus spp and determine the antibiotic resistance profile of isolated bacteria from commercial poultry carcasses in Ardabil province, northwest of Iran.

Materials and Methods

Sample collection

The study was carried out on 125 carcasses obtained from 30 commercial poultry flocks (including 27 broiler flocks, 1 laying flocks and 2 breeder flocks) in different parts of Ardabil province. So that, five carcasses were randomly selected from each flock and after necropsy, the heart blood and liver's visceral surface were sampled next to the flame. Collected samples were transported to the laboratory for Streptococcus spp isolation.

Identification of *Streptococcus spp*

Sample collected using cotton swab and stored in sample tube contained phosphate buffered saline (PBS). then the samples stored in cool box and the temperature was maintained in 4 ℃.
To isolate *Streptococcus spp*., sample cultured in blood agar (BA), then incubated 37 ° C for 18-24 hours. Then the results of a single colony that grows in culture in tryptone soya agar (TSA), incubated 37 ° C for 18-24 hours. Next, isolates were carried out Gram staining to determine bacterial morphology. Coloring results with The gram ositive morphology of cocci was then tested using catalase dripping 3% H2O2 in a few bacterial isolates [3, 4].

Antibiotic Susceptibility Test

To determine the antibiotic resistance of the isolates against 10 drugs commonly in the veterinary and medical fields was used qualitative disc diffusion method by Kirby Bauer procedure on Mueller-Hinton agar (Merck, Germany) in according with instructions of Clinical Laboratory Standards Institute Guidelines (CLSI) 2018. On this criterion, the organisms were classified as Resistant (R), Intermediate (I) or Susceptible (S).
Antibiotic disks, procured from Padtan Teb Company of Iran, were Ampicillin (10), Doxycycline (30), Clindamycin (30), Enrofloxacin (15), Gentamicin (10), Ciprofloxacin (5), Erythromycin (10), Chloramphenicol (30), Neomycin (30) and Cefatoxaime (5).

Result

In this study, ninety-eight (98) bacteria isolates were obtained from 125 carcasses while no bacteria were found in 34 carcasses. Out of 98 isolates, 6 (6.13 %) isolates were detected as Streptococcus spp. The frequency of resistance of Streptococcus spp isolates to ten antimicrobial agents is is as follows:
All isolates were resistant to doxycycline (100 %) and ampicillin (100 %). In 7 antibiotics, the highest resistance was found against Neomycin (83.4%) and Enrofloxacin (83.4%), followed by Erythromycin (66.7%), Gentamycin (50.0%), Ciprofloxacin (33.4%) Chloramphenicol (33.4%), Clindamycin (16.7%), respectively. No resistance to Cefatoxaime was observed within the isolate population.

Discussion

The *Streptococcus* genus consists of 104 recognized species, ranging from commensals to highly virulent pathogens. These Gram-positive bacteria are widespread in the environment, including water, soil, and the surfaces, digestive tracts, and respiratory systems of animals and humans [2, 4].
Prevalence of *Streptococcus spp* (6.13%) was observed in the current work. While Hermana et al., (2020) reported a higher prevalence (8%) [3]. In this study, *Streptococcus spp*. was resistant to ampicillin (100%) and doxycycline (87.5%) and the resistance to 7 other antibiotics ranged from 83.4 to 16.7 %. There are no resistant for Cefatoxaime for *Streptococcus spp*. *Streptococcus spp* from broiler isolates in chicken farm in West Java had experienced resistance to ampicillin (100%), chloramphenicol (87.5%), and doxycycline (0%) [3]. Similar research in Hungary mentioned *Streptococcus spp*. isolated from clinical cases of Waterfow were resistant to the antibiotic doxycycline 89.5%, amoxicillin 42.1%, enrofloxacin 26.3% and chloramphenicol 92.2% [4].
In this study, 83.4 % of isolates showed resistance against neomycin and enrofloxacin.These two antibiotics are most commonly used in poultry husbandry for prophylactic, metaphylactic and therapeutic purposes. In this study less resistance by these isolates to ciprofloxacin , chloramphenicol, clindamycin and cefatoxaime was observed as 33.4%, 33.4% , 16.7 % and o%. The reason behind less resistance of four antibiotics is not being used or very little of common use in the poultry industry of Iran.

Conclusion

In this study, 6 isolates were identified as *Streptococcus spp*. High resistance was observed against doxycycline, neomycin and enrofloxacin which are most commonly used in poultry industry. Isolates were the most susceptible to cefatoxaime and clindamycin. Therefore, performing antimicrobial sensitivity test is necessary prior to prescribing any antibiotic in commercial poultry

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