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Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from commercial poultry carcasses in Ardabil province, Iran

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Abstract

Poultry especially broiler constitutes a large part of protein production in Iran. Nonetheless, farmers use antibiotics for prevention, treatment, and growth enhancement. The objective of this investigation was to determine antibiotic resistance patterns of *S. aureus* isolated from commercial poultry carcasses in Ardabil province, northwest of Iran. Samples were taken from heart blood and liver's visceral surface of 125 carcasses obtained from 30 flocks (including 27 broiler flocks, 1 laying flocks and 2 breeder flocks) located in different parts of Ardabil province. The isolates of *S. aureus* were determined based on microbiological standard methods. The antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method. 98 bacteria isolates were identified from 125 carcasses while no bacteria were found in 34 carcasses. Out of 98 isolates, 8 (8.16%) isolates were detected as Staphylococcus aureus. The S. aureus isolates showed resistance to Doxycycline (100 %), Ampicillin (87.5%), followed by Methicillin (75.0%), Sulfamethoxazole-trimethoprim (62.5%), Ciprofloxacin (62.5%), Erythromycin (50.0%), Chloramphenicol (37. 5%), Gentamicin (37.5%), Vancomycin (25.0%) and Cefoxitin (12.5%). Our data indicated that antimicrobial resistance of *S. aureus* was in poultry flocks of Ardabil province, northwest of Iran, and that antibiotics, especially tetracycline and ampicillin, to treat in poultry should be used with caution.

Key Words: Antibiotic resistance, Poultry carcasses, S. aureus, Ardabil province

Introduction

Staphylococcus aureus is one of the most prevalent opportunistic pathogens and causes invasive and indubitably life-threatening infections (Amoako et al., 2020). In humans, it is the 3rd most common cause of food poisoning (Amoako et al., 2020). Infections caused by *S. aureus* have been reported in different type of meat like raw chicken, turkey, veal, beef, pork, lamb, and rabbit all over the world. *S. aureus* was found to be most prevalent in turkey followed by chicken, veal, pork, and beef 35.3%, 16.0%, 15.2%, 10.7% and 10.6% ,respectively in Netherland (De Boer et al., 2009). While in Germany, its prevalence was reported in 37.2% of samples of poultry food products (Feßler et al., 2011). In the USA, it was detected in 42.1% of poultry samples (Abdalrahman et al., 2015). From Asia, only a few reports are available, and mainly these reports are of livestock-associated *S. aureus* infections (Chuang et al., 2015). S. aureus is the prominent cause of many infections in the chicken like synovitis, osteomyelitis, and cellulitis (Amoako et al., 2020). It is renowned for its ability to develop resistance to antibiotics. In addition to this, antimicrobial resistance is causing financial loss to the poultry industry because poultry birds have developed resistance to antibiotics. Ample use of antibiotics in animal farming is the prime cause of the prevalence of drug resistance among foodborne pathogens mainly in *Salmonella*, *Staphylococcus spp.* and *E. coli* (Amoako et al., 2020; Ali et al., 2017; Vickers, 2017). It is a widely accepted notion that antibiotics provided in the diet generate selective pressure on the microbial flora that in return facilitates the persistence transfer of antimicrobial resistance determinants among bacterial species which leads to the emergence of multi-drug resistant bacteria (Amoako et al., 2020; De Boer et al., 2009; Suleiman et al., 2013).

Many researchers have isolated (MRSA) from poultry because of the excessive use of antibiotics in the poultry sector. Despite the fundamental consequences of AMR in poultry, our knowledge about antibiotic resistance in *S. aureus* in commercial poultry of Iran is rudimentary, ergo comprehensive research is needed. The present study is aimed to determine the prevalence and antibiotic resistance of *S. aureus* isolated from isolated 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 *S. aureus* isolation.

Identification of *S. aureus*

For processing, enrichment of each sample was carried out in buffer peptone water (BPW) and incubated at 37 °C for 24 h. Mannitol Salt Agar (MSA) was used as a selective and differential medium for the identification of *S. aureus*. For its purification purpose, distinct yellow colonies from MSA were streaked again onto MSA and stored in glycerol stocks. Initial identification was done based on colony morphology. Afterwards, Gram staining and biochemical characterization were carried out for complete identification which included catalase and coagulase tests.

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 amoxicillin (10), doxycycline (30), Vancomycin (30), Sulfamethoxazole-trimethoprim (30), gentamicin (10), ciprofloxacin (5), Erythromycin (10 μg), chloramphenicol (30), Cefoxitin (30) and Methicillin (10 μg).

Result

In this study, 98 bacteria isolates were obtained from 125 carcasses while no bacteria were found in 34 carcasses. Out of 98 isolates, 8 (8.16%) isolates were detected as *S. aureus*. The frequency of resistance of *S. aureus* isolates to ten antimicrobial agents is is as follows:

All isolates were resistant to to doxycycline (100 %) and the resistance to the 9 other antibiotics ranged from 87.5 to 12.5 %. In 9 antibiotics, the highest resistance was found against Ampicillin (87.5%), followed by Methicillin (75.0%),Sulfamethoxazole-trimethoprim (62.5%), Ciprofloxacin (62.5%), Erythromycin (50.0%) and the lowest resistance was found in Chloramphenicol (37. 5%), Gentamicin (37.5%), Vancomycin (25.0%) and Cefoxitin (12.5%).

Discussion

Staphylococcosis caused by S. aureus have varying degrees of morbidity and mortality. It has been reported as a leading pathogen in the poultry industry with a 38.5 % average disease prevalence in 21 European countries (Vickers, 2017). Prevalence of *S. aureus* (8.2%) was observed in the current work While Suleiman et al., (2013) and Ali et al., (2017) reported a higher prevalence (52% and 28%, respectively) [6, 8].

In this study, among all the samples of *S. aureus*, 100 %, 62.5 % and 50 % of isolates showed resistance against doxycycline, Sulfamethoxazole-trimethoprim and Erythromycin, respectively and a similar trend was shown by Yin et al., (2010); Agyare et al., (2018). These three antibiotics are most commonly used in poultry husbandry for prophylactic, metaphylactic and therapeutic purposes. Among all of these isolates 75% were resistant to methicillin and a similar trend was shown by Ali et al., (2017). The reason behind the more prevalence of MRSA might be due to the more common use of methicillin and oxacillin for treatment and growth purposes. In this study less resistance by these isolates to Chloramphenicol; Gentamicin, Vancomycin, and Cefoxitin was observed as 37.5%, 37.5% , 25% and 12.5%. 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, from a total of 98 isolates, 8 isolates were identified as *S. aureus*. High resistance was observed against Doxycycline, Ampicillin, Methicillin, Sulfamethoxazole-trimethoprim, Ciprofloxacin and Erythromycin with 100 %, 87.5%, 75.0%, 62.5%%,62.5% and 50.0% respectively. Isolates were the most susceptible to Cefoxitin, Vancomycin , Gentamicin and Chloramphenicol. Among isolates, only 75 % of isolates were found to be MRSA. The development of drug resistance by *S. aureus* is a matter of concern for food safety because this resistance is transferred from animals to humans. Surveillance of an antibiogram of *S. aureus* is essential for one health concept.

References

Amoako, D.G., Somboro, A.M., Abia, A.L., Molechan, C., Perrett, K., Bester, L.A., & Essack, S.Y. (2020). Antibiotic resistance in *Staphylococcus aureus* from poultry and poultry products in uMgungundlovu District, South Africa, using the Farm to Fork approach. Microbial Drug Resistance, 26(4), 402-411. https://doi.org/10.1089/mdr.2019.0201

De Boer, E., Zwartkruis-Nahuis, J.T., Wit, B., Huijsdens, X.W., De Neeling, A.J., Bosch, T., Van Oosterom, R.A., Vila, A., & Heuvelink, A.E. (2009). Prevalence of methicillin-resistant *Staphylococcus aureus* in meat.

International journal of food microbiology, 134(1-2), 52-56. https://doi.org/10.1016/j.ijfoodmicro.2008.12.007

Feßler, A.T., Kadlec, K., Hassel, M., Hauschild, T., Eidam, C., Ehricht, R., Monecke, S., & Schwarz, S. (2011). Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. Applied and environmental microbiology, 77(20), 7151-7157. https://doi.org/10.1128/AEM.00561-11

Abdalrahman, L.S., Stanley, A., Wells, H., & Fakhr, M.K. (2015). Isolation, virulence, and antimicrobial resistance of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA) strains from Oklahoma retail poultry meats. International journal of environmental research and public health, 12(6), 6148-6161. https://doi.org/10.3390/ijerph120606148.

Chuang, Y.Y., & Huang, Y C. (2015). Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: an emerging issue?. International Journal of Antimicrobial Agents, 45(4), 334-340. https://doi.org/10.1016/j.ijantimicag.2014.12.007.

Ali, Y., Islam, M,A., Muzahid, N.H., Sikder, M.O., Hossain, M.A., & Marzan, L.W. (2017). Characterization, prevalence and antibiogram study of *Staphylococcus aureus* in poultry. Asian Pacific Journal of Tropical Biomedicine, 7(3), 253-256. https://doi.org/10.1016/j.apjtb.2016.12.001

Vickers, N.J. (2017). Animal communication: when i'm calling you, will you answer too?. Current biology, 27(14), R713-R715. https://doi.org/10.1016/j.cub.2017.05.064

Suleiman, A., Zaria, L.T., Grema, H.A., & Ahmadu, P. (2013). Antimicrobial resistant coagulase positive *Staphylococcus aureus* from chickens in Maiduguri, Nigeria. Sokoto Journal of Veterinary Sciences, 11(1), 51-55. https://doi.org/10.4314/sokjvs.v11i1.8

Yin, Y., Lei, F., Zhu, L., Li, S., Wu, Z., Zhang, R., Gao, G.F., Zhu, B., & Wang, X. (2010). Exposure of different bacterial inocula to newborn chicken affects gut microbiota development and ileum gene expression. The ISME journal, 4(3), 367-376. https://www.nature.com/articles/ismej2009128

Agyare, C., Boamah, V.E., Zumbi, C.N., Osei, F.B. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. Antimicrobial resistance—A global threat, 33-51. https://doi.org/10.5772/intechopen.79371