SHORT COMMUNICATION

Prevalence, Virulence Traits and Antimicrobial Resistance Pattern of *Staphylococcus aureus* Isolated from Chicken

Amruta Doiphode[1]*, Vikas Waskar[1], Rahul Kolhe[1], Bapurao Kadam[2] and Prashant Mhase[3]

Abstract

Staphylococcus aureus isolated from chicken samples of retail market of Shirwal city exhibited 36% (18/50) prevalence, confirmed biochemically as well as by polymerase chain reaction by employing 16s-rDNA and species-specific sau genes. None of the isolates were found to possess virulence genes, viz., *sea, seb, sec* and *sed*. Antimicrobial resistance pattern revealed that 100% isolates were resistant to 16 among 24 antibiotics, while 5 antibiotics showed more than 70% resistance, except for tobramycin (44.44%) and gentamicin, streptomycin (38.89% each). All isolates were multidrug resistant (MDR). Screening for the presence of antimicrobial resistance genes revealed the presence of *aacA-D, ermA, tetK* and *tetM* genes. None of the isolates carried *mecA, mrsA, mrsB, vanA, vanB* and *ermC* genes, although phenotypic resistance was noted.

Keywords:Antimicrobial resistance, Chicken, Prevalence, *Staphylococcus aureus*, Polymerase Chain Reaction (PCR), Virulence Genes

Ind J Vet Sci and Biotech (2022): 10.48165/ijvsbt.18.4.26

Introduction

Food-borne disease outbreaks remain a major global health challenge and cross-contamination from raw meat due to poor handling is a major cause in developing countries. *S. aureus*, a ubiquitous food-borne pathogen, may contaminate food materials and is also responsible for the development of antimicrobial resistance (Baghbaderani et al., 2020).

Many investigations have shown that *S. aureus* is resistant to many antibiotics and carries many antimicrobial-resistant genes. Antimicrobial resistance (AMR) has been declared by the World Health Organization as one of the top 10 global public health threats for humans (WHO, 2021). World Health Organization has categorized antibiotic-resistance pathogens into critical, high and medium priority groups for research and development of new antibiotics. Methicillin-resistant and vancomycin-intermediate and resistant *Staphylococcus aureus* is included in the second category as a high priority pathogen (WHO, 2017). *S. aureus* in the food chain is considered as an indicator of bad and poor hygiene; hence the presence of organisms and their exotoxins in food is considered a source of food poisoning (FDA, 2016). Meat derived from poultry reared under intensive management conditions may get contaminated during slaughter procedures due to unhygienic handlings adopted in wet market. It has been well documented that *S. aureus* shows resistance to many antibiotics and carries multiple antimicrobial resistance genes as well as virulence genes. This study aimed to investigate the prevalence of *S. aureus* and its virulence traits, antimicrobial resistance pattern and antimicrobial genes in chicken samples of retail market of Shirwal, Maharashtra (India).

[1]Department of Veterinary Public Health, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara-412801, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur-440001, India.

[2]Department of Livestock Products Technology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara-412801, MAFSU, Nagpur-440001, India.

[3]Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara-412801, MAFSU, Nagpur-440001, India.

Corresponding Author: Amruta Doiphode, Department of Veterinary Public Health, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara-412801, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur-440001, India, e-mail: amruta1124@gmail.com.

How to cite this article: Doiphode, A. Waskar, V., Kolhe, R. Kadam, B. & Mhase, P., Prevalence, Virulence Traits and Antimicrobial Resistance Pattern of Staphylococcus aureus Isolated from Chicken (2022). Ind J Vet Sci and Biotech. 18(4),130-132.

Source of support: Nil

Conflict of interest: None.

Submitted: 12/01/2022 **Accepted:** 23/08/2022 **Published:** 10/09/2022

MATERIALS AND METHODS

Isolation and Identification of *S. aureus*

Specimens from chicken were collected from 50 different retail shops located in 5 different villages in and around Shirwal, Dist. Satara (Maharashtra, India). The samples were analyzed for the presence of S. *aureus* as per the

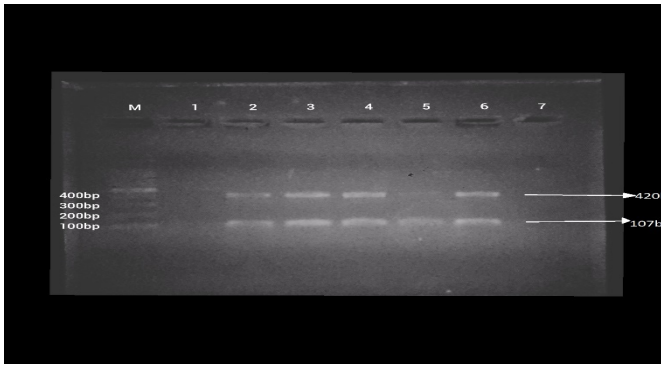

Fig. 1: Amplification of 16S-rDNA and sau gene

for *vanA* and *vanB* genes.

RESULTS AND DISCUSSION

Biochemically confirmed isolates from 50 chicken specimens revealed, 18 (36%) positive samples of *S. aureus,* which was similar to Patyal *et al.* (2012), Govender et al. (2019), Savariraj *et al.* (2020) and Tegegne *et al.* (2021).

Molecular characterization of isolates by targeting 16S-rDNA and sau genes, confirmed only 4/50 (8%) samples as S. aureus (Fig. 1). Upon characterization of isolates from chicken for virulence genes, it was noticed that none of the isolates harbored virulence genes. Shylaja *et al.* (2018), Savariraj *et al.*

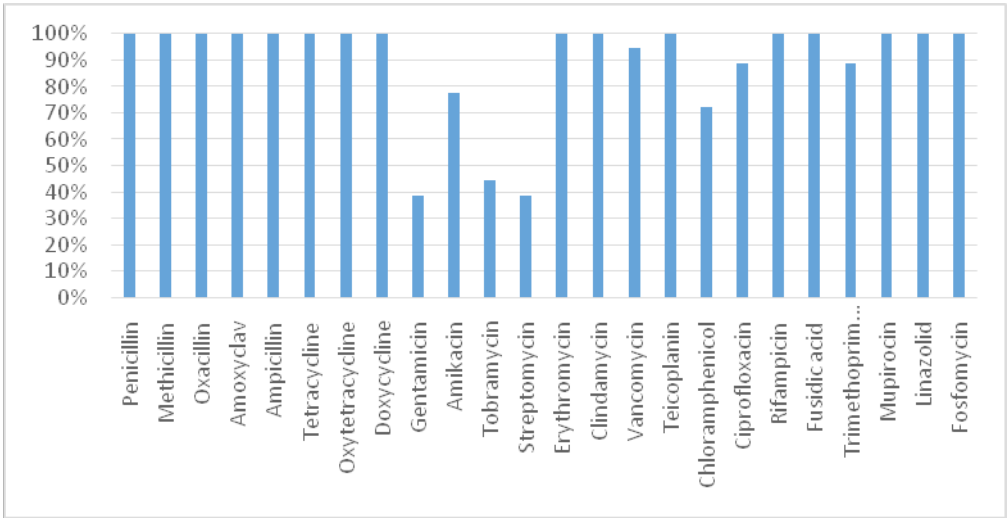

Fig. 2: Antimicrobial resistance pattern of *S. aureus* isolates

Bacteriological Analytical Manual (FDA, 2019) with slight modifications. Presumptive isolates were further subjected to biochemical characterization using catalase test, DNA-ase test (Cheesebrough, 2006) and Voges-Prausker test, methyl red test and oxidase test (Cruickshank *et al.,* 1975).

Antibiogram

Antibiogram sensitivity and resistance pattern of *S. aureus* was interpreted using the Kirby Bauer disc diffusion method (Bauer *et al.,* 1966) and the results were recorded following the guidelines given by the Clinical and Laboratory Standards Institute (CLSI, 2018).

PCR Characterization of S. aureus for Virulence Genes and Antimicrobial Resistance Genes

All biochemically confirmed and DNA-ase positive isolates were subjected to a polymerase chain reaction for characterization of various genes, viz., 16s- rDNA and sau genes as per Strommenger *et al.* (2003); virulence genes (sea, seb, sec and sed) according to Mehrotra *et al.* (2000) and antibiotic resistance genes using protocols of Kumar *et al.* (2009) for detection of mecA, tetK, tetM, ermA, ermC, aacA, mrsA and mrsB genes and as per Hizlisoy *et al.* (2018) (2020) and Parvin *et al.* (2021) also reported lower prevalence.

Antimicrobial resistance pattern (Fig. 2) revealed that S. aureus isolates showed high resistance to ß lactamase group of antibiotics. 100% resistance has been shown to penicillin, ampicillin, oxacillin, methicillin, amoxicillin-clavunalic acid, tetracycline, oxytetracycline, doxytetracycline, erythromycin, clindamycin, teicoplanin, rifampicin, fusidic acid, mupirocin, fosfomycin, linezolid. While 94.44% for vancomycin, 88.89% for ciprofloxacin and trimethoprim-sulphamethoxazole and 72.22% for chloramphenicol. Comparatively, low resistance was shown for tobramycin, which was 44.44%, while 38.89% resistance was for gentamicin and streptomycin.

A similar antibiotic resistance pattern was noted by Akbar and Anal (2013), Duran *et al.* (2017), Herve and Kumar (2017), Ruban *et al.* (2018), Govender *et al.* (2019) and Parvin *et al.* (2021). Interaction with field veterinarians and pharmacists in the study area revealed that clindamycin, mupirocin, rifampicin, fusidic acid and fosfomycin preparations are not prescribed for veterinary use in the study area, however, these antibiotics are commonly used in human practice. Antimicrobial-resistant bacteria may get transferred between humans and animals through a shared environment (Woolhouse *et al.,* 2015), hence chances of cross-contamination cannot be ruled

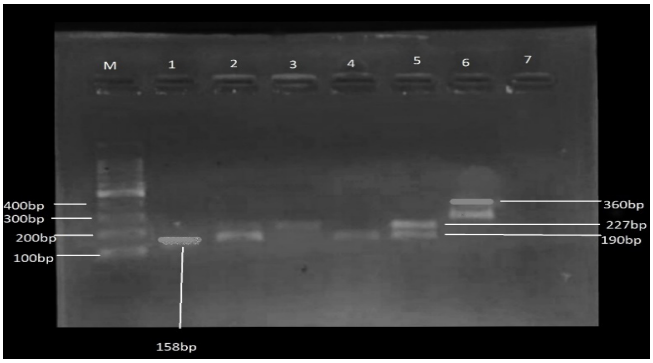

Fig. 3: Gel electrophoresis of antimicrobial resistance genes
M- Ladder (100bp), Lane1 – tetM (158bp)
Lane 2 and 5- ermA (190bp), aacA-D (227bp) Lane 3- aacA-D

out. All isolates were multidrug-resistant showing resistance to more than 3 antibiotics, 5 isolates showed resistance to 16-20 antibiotics and 13 isolates showed resistance to 21-24 antibiotics. Indiscriminate use of antibiotics could be attributed to such a high multidrug resistance rate.

Screening for antimicrobial resistance genes revealed that the prevalence of *aac*A-D was 55.55% followed by *tetM* (44.44%) , *ermA* (38.89%) and *tetK* (11.11%) for, while *mecA*, *vanA, vanB, mrsA, mrsB* and *ermC* were not observed in any of the isolates (Fig. 3). Some isolates have shown phenotypic resistance but were unable to express antimicrobial genes, may be due to alternative mechanisms or genes responsible for resistance. Similar findings were also observed by Zehra *et al*. (2019), Fazel *et al*. (2020) and Baghbaderani *et al*. (2020).

CONCLUSION

Adherence to good meat hygienic practices should be followed to prevent contamination of chicken. Avoiding indiscriminate use of antibiotics and good hygienic practices are necessary to control antimicrobial resistance.

ACKNOWLEDGEMENT

We are grateful to the Associate Dean of the Veterinary College, Shirwal and the authorities of MAFSU, Nagpur for the facilities provided for this work.

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