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Antimicrobial resistance profile of *Staphylococcus aureus* from clinical and non-clinical sources in selected regions of Bareilly, India

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Abstract
Objective: The research was structured to investigate the resistance patterns of *S. aureus* isolates from Bareilly, India, particularly concerning tetracycline and quinolone resistance.
Methods: Standard microbiological techniques were utilized to isolate and characterize the *S. aureus* strains. Isolates were subjected to ABST. PCR was employed to detect and characterise the genes associated with tetracycline and quinolone resistance.
Results: Around 164 isolates of staphylococci isolated from mastitic milk, wound, and street food samples were used for the study. Out of 164 isolates, 77 (46.9%) were confirmed as *S. aureus*. Among 77 *S. aureus* isolates, 14% and 43% isolates were resistant to doxycycline and ciprofloxacin, respectively. Almost 50.6% of *S. aureus* isolates were multidrug resistant. Genotypic detection of AMR in *S. aureus* isolates revealed that 47%, 11.7% and 5.8% of doxycycline resistant isolates were positive for *tet*K, *tet*M and *tet*L genes. The *tet*M gene was significantly more common in *S. aureus* causing wound infections while no significant difference was apparent for occurrence of *tet*K and *tet*L carrying *S. aureus* of different origin.

Keywords: Multidrug resistance, tetracyclines, quinolones, *S. aureus*, India, clinical samples

Introduction
Bacterial antimicrobial resistance (AMR) represents a significant challenge to global health. The worldwide impact of drug-resistant infections resulted in approximately 4.95 million deaths, with 1.27 million directly linked to drug resistance. In 2019, *S. aureus* was identified as one of the six major pathogens contributing to the burden of AMR (Murray *et al*., 2019) [6]. Therefore, this study was undertaken to assess the antimicrobial susceptibility of *S. aureus* isolates to commonly prescribed antibiotics like doxycycline and ciprofloxacin.

Materials and Methods
A total of 80 samples, including 55 mastitic milk samples, 20 wound swabs, and 5 street food samples, were collected and appropriately labeled. These samples were transported to the Clinical Laboratory of the Epidemiology Division of the Indian Veterinary Research Institute (located in Bareilly, India). Within 6 hours of collection, the samples were processed to isolate *S. aureus*. They were inoculated into tryptic soy broth containing 7.5% NaCl and incubated at 37 °C for 24 hours. Following incubation, overnight cultures were streaked onto mannitol salt agar. Yellow-colored colonies typical of *S. aureus* were selected from the mannitol salt agar and streaked onto nutrient agar. The characteristic colonies grown on the nutrient agar were subjected to Gram staining and biochemical tests including Catalase, Coagulase test for further characterization.
The isolates underwent antibiotic susceptibility testing against a panel of antibiotics including doxycycline (DO-30 µg), tetracycline (TE-30 µg), ciprofloxacin (CIP-5 µg), enrofloxacin (ENO-5 µg), gentamicin (GEN-10 µg), azithromycin (AZM-15 µg), chloramphenicol (C-30 µg), nitrofurantoin (NIT-300 µg), sulpha/trimethoprim (COT-25 µg), cefoxitin (CX-30 µg), oxacillin (OX-1 µg), vancomycin (VA-30 µg), rifampicin (RF-5 µg), clindamycin (CD-2 µg),

linezolid (LZD-30 µg), and mupirocin (MU-200 µg). The interpretation of antimicrobial susceptibility results was based on criteria established by the Clinical and Laboratory Standards Institute (CLSI, 2018) [3].

Genomic DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and polymerase chain reaction (PCR) was conducted in a final reaction volume of 25 µL. Duplex PCR was utilized to identify the *S. aureus* specific sequences of nuc (Brakstad *et al*., 1992) [1] and 23SrRNA (Shome *et al*., 2011) [7] using control strains of *S. aureus* (ATCC 43300) and (ATCC 29213). To remove the chances of contamination in PCR reactions, a NTC (non-template control) was prepared and run in every PCR reaction. The PCR products were separated on 1.5% agarose gel by gel electrophoresis. All phenotypically confirmed *S. aureus* isolates underwent PCR amplification for the identification of tetracycline resistance genes, tetK, tetM, and tetL. The details of the primers, their respective annealing temperatures, and amplification sizes are provided in Table 1. Positive PCR amplicons were sent to commercial sequencing services (Eurofins Ltd, Bangalore) for further purification and sequencing using Sanger's dideoxy method.

Table 1: Details of the primer sequence, annealing temperature and product size for *S. aureus* isolates

Gene		Primer Sequence (5'-3')	Annealing Temperature (°C)	Product size (bp)	Reference
nuc	F	GCGATTGATGGTGATACGGT	52	279	Brakstad *et al*.,1992 [1]
	R	AGCCAAGCCTTGACGAACTAAAGC			
*23Sr*RNA	F	AGCGAGTCTGAATAGGGCGTTT	52	894	Shome *et al*., 2011 [7]
	R	CCCATCACAGCTCAGCCTTAAC			
*tet*K	F	GTAGCGACAATAGGTAATAGT	55	360	Warsa *et al*., 1996 [9]
	R	GTAGTGACAATAAACCTCCTA			
*tet*M	F	AGTGGAGCGATTACAGAA	55	158	Warsa *et al*., 1996 [9]
	R	CATATGTCCTGGCGTGTCTA			
*tet*L	F	ATAAATTGTTTCGGGTCGGTAAT	55	1077	Warsa *et al*., 1996 [9]
	R	AACCAGCCAACTAATGACAATGAT			

Results and Discussion

A total of 164 *Staphylococcus* spp. isolates were obtained from 80 samples, with 96 isolates displaying distinct biochemical characteristics. Among these 96 isolates, 73 were identified as coagulase positive. Similarly, Vishnu Ravichandran (2021) [8] reported that 26 of the 29 *S. aureus* were phenotypically positive for coagulase test. However, coagulase positive *S. aureus* could not always be detected phenotypically because some of the biochemical characteristics were weak or unusual for certain species. All the 96 isolates were subjected to duplex PCR assay for confirmation of *S. aureus* by targeting nuc gene and 23S rRNA sequences and 77 isolates were found to be positive for *nuc* gene indicating *S. aureus*. However, only 73 isolates possessed *S. aureus* specific 23S *rRNA* gene. Brakstad *et al*., (1992) [1] reported that identification of *nuc* gene is enough for *S. aureus* identification.

Among various source of samples, *S. aureus* detection rate was highest in mastitic milk (70.12%) followed by wound swabs (23.37%) and street food samples (6.49%). The difference may be due to variations in sample size, environmental & managemental factors.

Among 77 isolates of *S. aureus*, Highest number of isolates were resistant to cefoxitin (49%) followed by ciprofloxacin (43%) and tetracycline (26%). However, low resistance rate were reported for chloramphenicol (9%) and nitrofurantoin (9%). Among 11 doxycycline resistant isolates, 5 and 6 isolates were from mastitic milk and wound swabs, respectively. Of the 33 ciprofloxacin resistant isolates, 4, 14 and 15 isolates were from street food, wound and mastitic milk samples, respectively. A total of 38 (49.3%) *S. aureus* isolates exhibited MDR among which 20, 14 and 4 isolates were from mastitic milk, wound and street food samples, respectively.

Phenotypic tetracycline, doxycycline and ciprofloxacin resistant isolates were subjected to genotypic characterization by PCR detection. Among *S. aureus* isolates, 9, 2 and 1 isolate were positive for *tet*K, *tet*M and *tet*L gene, respectively. Among 9 *tet*K positive isolates, 6, 2 and 1 isolates were from mastitic milk, wound and street food sample, respectively. The *tet*M gene was significantly more common in *S. aureus* causing wound infections while no significant difference was apparent for occurrence of *tet*K and *tet*L carrying *S. aureus* of different origin. Zehra *et al*., (2018) [10] from India reported *tet*K, *tet*M and *tet*L genes from meat samples. Fawzy *et al*., (2017) [5] reported *tet*K gene in 8 tetracycline resistant isolates of *S. aureus*. Diab *et al*., (2021) [2] reported *tet*K gene which was frequently detected with overall prevalence of 35%. Emaneini *et al*., (2013) [4] reported 61% tetracycline resistant *S. aureus* among which 32.4% were carrying *tet*M gene and 17.2% carrying *tet*K gene. Among the isolates tested 13.9% isolates showed coexistence of tet*M* and tet*K*.

The present study revealed occurrence of antimicrobial resistance with main focus on tetracycline and ciprofloxacin resistance since those are most commonly used antibiotics for veterinary treatment.

Conclusion

The study highlights the Tetracycline and quinolone resistance pattern of *S. aureus* isolates from samples of different origin. Tetracyclines and quinolones are most commonly used antibiotics for treatment of animals in veterinary practice. Hence, necessary steps are needed for judicious use of commonly used antimicrobials to decrease the prevalence of AMR.

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