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Antibiogram study of *Staphylococcus aureus* isolates from buffaloes with subclinical mastitis

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Abstract
In subclinical mastitis *Staphylococcus aureus* (*S. aureus*) is a prominent bacterium. Resistance to antibiotics is a serious concern when confronted with these kind of infectious agent. In our investigation, 41 of 84 milk samples confirmed positive for bacterial culture and genotypically confirmation done by targeting 23S rRNA. In our investigation, the best-performing effective antibiotics for *S. aureus* comprised chloramphenicol (87.80%) along with tetracycline (78.04%) afterwards methicillin, and ceftriaxone (75.60%), while the rest of the antibiotics had lesser efficacy. *S. aureus* had the greatest resistance toward both antibiotics including penicillin-G and Ampicillin (100% each). This study highlights diversified antimicrobial resistance of *S. aureus* against wide variety of antibiotics. Routine antibiotic resistance surveillance is required to address this issue.

Keywords: Resistance to antibiotics, buffaloes, *Staphylococcus aureus*, subclinical mastitis

Introduction
Mastitis constitutes a serious buffalo disease that causes substantial financial losses primarily owing to poor milk productivity, deteriorated milk quality, treatment costs, and so on. Subclinical mastitis is a form of mastitis which remains hidden in dairy herd. The resistance to antibiotics emerges in multiple pathogenic strains of bacteria as a consequence of repeated use it is critical to understand the bacteria's resistance before beginning chemotherapy. Antimicrobial resistance acquisition in *S. aureus* strains is a major source of worry in the milk industry (Wang *et al.*, 2008) [29], as it gains resistance to antibiotics with extraordinary efficiency (Booth *et al.*, 2001) [7]. *S. aureus* developed penicillin resistance by producing a specialised enzyme termed penicillinase (β-lactamase), which is expressed by a *blast* gene found in plasmids. Lowy (2003) [12] describes this enzyme penicillinase is responsible for breakdown the β-lactam ring of penicillin antibiotics. This study was conducted to assess the susceptibility of *S. aureus* isolates from buffaloes suffering subclinical mastitis to various antimicrobial agents.

Materials and Methods
Collection of sample: A combined collection of 84 physically typical milk samples (about 5-10 ml from every sample) was taken across 84 buffaloes owned by various farmers located near Hanumangarh (The Rajasthan State). All of the samples have been taken in disinfected test containers and promptly sent to the facility under ice for additional examination.

Isolation of *Staphylococcus aureus* and genotypic confirmation: The DNA from bacteria has been separated using the procedure described by Nachimuttu *et al.* (2001) [13], with minor changes. The genotypic verification was performed employing 23S rRNA gene based ribotyping as revealed by Straub *et al.* (1999) [32], with the primers listed in Table 1.

Table 1: Primers employed for genotypic authentication of *S. aureus*

S. No.	Target Gene	Primer sequence	Size (bp)	Reference
1	23S rRNA	F: 5´-ACGGAGTTACAAAGGACGAC-3´	1250	(Straub *et al.*, 1999) [32]
		R: 5´-AGCTCAGCCTTAACGAGTAC-3´		

The mixture used for the reaction (25 µl.) was manufactured by combining the following components in Table 2.

Table 2: Components of PCR mixture

Genetaq Green Master Mix,2X	12.5 µl
Primer-F(25 pM/µl)	0.5 µl
Primer-R(25 pM/µl)	0.5 µl
DNA template	3.0 µl
Nuclease free water to make	25.0 µl

Antibiotic sensitivity evaluation

The Bauer *et al.* (1966) [5] methodology was used to figure out the isolates' antibiograms against various antibiotics. In brief, the bacterial isolates were placed into a disinfected 5 ml nutrient broth container, cultivated for 18 hours at 37°C, and then calibrated to 0.5 McFarland turbidity norms (Quinn *et al.* 2000) [17]. The suspension of bacteria was evenly distributed over the agar plate using a sterilized swab. Screens have been dried for 10 minutes at 37°C before carefully putting antibiotic discs over the surface, leaving adequate space for the antibiotic to diffuse. Plates were subjected to incubation at 37°C for 24 hours, and the extent of restriction of growth surrounding each of the discs was calculated in millimetres and compared to the recommended chart that was supplied by the disc supplier.

Results and Discussion

Genotypic characterization using the 23S rRNA gene

Although *S. aureus* can be recognized by its numerous phenotypic traits, in the current study, genotypic verification was also performed using a PCR-based technique employing a unique primer directed for the 23S rRNA sequence. Using the particular primer, an amplified fragment of 1250 bp has been generated across all 41 buffalo subclinical mastitis isolates that were unique to *S. aureus*. Various authors utilised a similar genotypic strategy for identifying *S. aureus* (Sanjiv *et al.*, 2008; Upadhyay *et al.*, 2010; Rathore and Kataria (2012) [19, 25, 18].

Antibiogram study: In the current study, 14 antibiotics of various types were employed to conduct an antibiogram analysis of all 41 isolates (Table 3). In our investigation, the best-performing effective antibiotics for *S. aureus* comprised chloramphenicol (87.80%) along with tetracycline (78.04%) afterwards methicillin, and ceftriaxone (75.60%), while the rest of the antibiotics had lesser efficacy. *S. aureus* had the greatest resistance toward both antibiotics including penicillin-G and Ampicillin (100% each).

Table 3: Antibiogram result of *S. aureus* isolates obtained from subclinical mastitis in buffaloes

S. No.	Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
1.	Chloramphenicol	87.80	07.31	04.87
2.	Tetracycline	78.04	14.63	07.31
3.	Ceftriaxone	75.60	14.63	09.75
4.	Methicillin	75.60	-	24.39
5.	Cefazolin	70.73	12.19	17.08
6.	Amoxicillin/clavulanic Acid	70.73	-	29.26
7.	Piperacillin-Tazobactam	70.73	-	29.26
8.	Gentamicin	63.41	21.95	14.63
9.	Ciprofloxacin	39.02	36.58	24.39
10.	Clindamycin	19.51	56.09	24.39
11.	Azithromycin	04.87	60.97	34.14
12.	Cefepime	04.87	19.51	75.60
13.	Ampicillin	-	-	100
14.	Penicillin-G	-	-	100

All *S. aureus* isolates from milk seemed resilient to penicillin-G (100%), and this is consistent with the findings of Nigam *et al.* (2015) [14], who found 100% resistance to penicillin-G toward *S. aureus* isolates from subclinical and clinical mastitis in buffaloes and cattle. Dan *et al.* (2019) [9] found that *S. aureus* isolates from clinical samples and milk were tolerant to penicillin-G at 91.7%. Kumar *et al.* (2010) [11] found 22.7% resistance to penicillin-G in *S. aureus* isolates, which is much lower than the results obtained in this investigation. Subclinical mastitis *S. aureus* isolates have shown 100% resistance toward ampicillin in present study. Similar resistance (97%) observed by Abed *et al.* (2018) [1] in *S. aureus* isolates from cow milk with subclinical mastitis and clinical. Contrary to our result Kumar *et al.* (2010) [11] recorded 84.4% sensitivity toward ampicillin for *S. aureus* isolates from cattle mastitis milk. In this study, 75.60% *S. aureus* isolates found sensitive toward ceftriaxone, however lower susceptibility was observed by Swarankar *et al.* (2017) [24] where 62.5% *S. aureus* isolate from buffalo mastitic milk showed resistance toward ceftriaxone. Similar lower sensitivity (69.2%) was recorded by Chandrasekaran *et al.* (2014) in clinical mastitis *S. aureus* isolates. We recorded 70.73% isolates to be sensitive toward piperacillin-tazobactam which is lower than that recorded by Sharma *et al.* (2015) [22] who recorded 88.89% of *S. aureus* isolates from subclinical and clinical mastitis of cattle sensitive to piperacillin-tazobactam. Contrary to this study, *S. aureus* isolates from subclinical and clinical mastitis of camel showed lower (54%) sensitivity towards piperacillin-tazobactam (Aqib *et al.*, 2017) [4]. In our study, we recorded 70.73% of *S. aureus* susceptible to cefazolin. In another study 33.33% susceptibility to cefazolin observed by Nigam *et al.* (2015) [14] which is much lower than present study. The clindamycin susceptibility pattern obtained in this study was 19.51% which is lower than the susceptibility (50%) reported by Vásquez-García *et al.* (2017) [27] in isolates from buffalo subclinical mastitis. Sharma *et al.* (2013) [21] reported (60%) susceptibility toward clindamycin.

Cefepime was shown to be resistant to 75.60% of *S. aureus* isolates isolated from buffaloes experiencing subclinical mastitis in this study. Vasquez-Gracia *et al.* (2017) [27] found 50% susceptibility to cefepime in *S. aureus* samples of bovine subclinical mastitis, which contradicted our findings. Cefepime was not recommended by the OIE for veterinary usage (Anonymous, 2015) [3]; this antibiotic is ineffective against *S. aureus*. We found 70.73% of isolates responsive to amoxicillin-clavulanic acid, which is consistent with Yadav *et al.* (2015a) [30] result that 75% of isolates were susceptible to amoxicillin-clavulanic acid. Pati and Mukharjee (2016) [15] discovered that amoxicillin-clavulanic acid was effective against a lower (52%) percentage of *S. aureus* isolated in bovine mastitis.

In the present study, 87.80% of *S. aureus* isolates have sensitivity towards chloramphenicol this result is showing similarity to the observations made by Bhati *et al.* (2013) [6] and Yadav *et al.* (2015b) [31], 100% of cattle subclinical mastitis *S. aureus* isolates were found sensitive. However, lower susceptibility (62.5%) was recorded by Akindele *et al.* (2010) [2] against *S. aureus* isolates from human clinical specimens. In present study sensitivity shown by *S. aureus* isolates toward ciprofloxacin was 39.02% which was much lower than that was 94% observed by Sanjiv and Kataria (2006) [20] against *S. aureus* isolates from cattle clinical mastitis. Verma *et al.* (2018) [28] recorded the sensitivity of *S. aureus* isolates toward ciprofloxacin to be 41.49% which is similar to that in present study.

In the current investigation, the sensitivity of S. aureus isolates to methicillin was 75.60%, which is consistent with the discovery presented by Sudhanthiramani *et al.* (2015) [23], who found 79.07% of S. aureus isolates from milk samples to be methicillin-sensitive. The *S. aureus* isolates from subclinical mastitis have shown 78.04% susceptibility toward tetracycline in the current investigation. Similar sensitivity of *S. aureus* isolates to tetracycline (81.39%) was reported by Sudhanthiramani *et al.* (2015) [23] in *S. aureus* isolates from milk samples. Contrary to our result Haque *et al.* (2018) [10] recorded only 9% *S. aureus* isolates to be sensitive toward tetracycline. Piotr *et al.* (2013) [16] recorded resistance toward tetracycline in 98.1% *S. aureus* isolates from a mastitic cow which is contrary to our result.

In present study resistance for *S. aureus* isolates toward Azithromycin recorded was 34.14% but Upadhyay and Kataria (2009) [26] revealed that the sensitivity of *S. aureus* isolates against azithromycin was 100% which is much higher than the observation made by us. We found that *S. aureus* specimens were 63.14% sensitive to gentamicin. Verma *et al.* (2018) [28] made an equivalent perception, identifying (65.96%) gentamicin-sensitive isolates. However, Sanjiv and Kataria (2006) [20] reported that all of the isolates (100%) exhibited responsive to gentamicin, which is significantly higher than in the current investigation.

Conclusion

Since this organism is more efficient in acquiring antibiotic resistance within very short period of its exposure to antibiotics, it is critical to assess antimicrobial resistance and vulnerability for the different generations of antibiotics that were previously used and are now in use. To ensure the health of human and animal together under one health approach, need of time is routine antibiotic resistance surveillance.

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