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Detection and analysis of antibiotic resistance variability among *Staphylococcus aureus* isolates from animal and human sources

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ABSTRACT

In consideration of the importance of *Staphylococcus aureus* regarding its contribution to antibiotic resistance, the present study was designed to find variability among *S. aureus* isolates in relation to their multidrug resistance patterns. A total of 157 species-specific 23S rRNA based confirmed *S. aureus* isolates from various clinical and non-clinical animal sources (cattle, buffalo, goat, sheep, dog, camel, pig and horse), human and pieces of meat from butcher shops were included in the present study. Overall more than 95% isolates were recorded resistant to ampicillin and penicillin-G, while approximately 100% isolates were sensitive to chloramphenicol, meropenem and nitrofurantoin. The isolates from different sources showed highly significant ($P \leq 0.01$) variation in their resistance patterns for 39 antibiotics, significant variation ($P \leq 0.05$) for levofloxacin and nitrofurantoin, and no significant variation ($P > 0.05$) for clindamycin. In Bonferroni correction, human isolates were significantly variable with a $P < 0.0001$ probability level of variance in relation to other pieces of meat and animal origin sources for most of the antibiotics. Human isolates had the highest (0.40) MAR index. A highly significant difference was observed in the antibiogram pattern between different sources of *S. aureus,* which may indicate the pattern and frequency of use of various antibiotics in humans and animals.

Key words: *Staphylococcus aureus;* antibiotic resistance; animal; human; DMRT analysis

Introduction

Staphylococcus aureus is a notorious member of the *Staphylococcaceae* family associated with various clinical and sub clinical infections. In humans, it may also cause serious infections, particularly in persons debilitated by chronic illness, traumatic injury, burns and immunosuppression. These infections include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, boils, furuncles, styes, impetigo, toxic shock syndrome and meningitis, and are often associated worldwide with hospitalized patients rather than healthy individuals in the community (CARTER et al., 1990). This organism is also known to cause a variety of suppurative infections, septicemia and

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toxinoses in domestic animals and/or birds, such as: mastitis, udder impetigo, endometritis, pyoderma, cystitis, dermatitis, botryomycosis of mammary glands, arthritis, scirrhous cord, bumble foot and tick pyaemia (NABER, 2009).

Although *S. aureus* is naturally susceptible to many antibiotics, it is acquiring antimicrobial resistance for multifactorial reasons that include the excessive widespread and inappropriate use of antimicrobials, the extensive use of these agents as growth enhancers in animal feed, and, with the increase in regional and international travel, the relative ease with which antimicrobial-resistant bacteria cross geographical barriers (LOWY, 2003). Staphylococci have developed many efficient mechanisms to neutralize new antibiotics, namely: enzymatic inactivation of the antibiotic, alteration of the target with decreased affinity for the antibiotic, trapping of the antibiotic, and efflux pumps. Complex genetic arrays have been acquired by *S. aureus* through horizontal gene transfer, while resistance to other antibiotics, including some of the most recent ones (*e.g.*, fluoroquinolones, linezolid and daptomycin), has developed through spontaneous chromosomal and plasmid mutations and antibiotic positive selection (LOWY, 2003; PANTOSTI et al., 2007).

In the course of resistance development, *S. aureus* showed resistance towards penicillin by the production of a specific enzyme called penicillinase (β-lactamase), often governed by genes located in plasmids. This is an extracellular enzyme, hydrolyzing the β-lactam ring of β-lactam antibiotics (penicillin) (LOWY, 2003). Methicillin resistant *S. aureus* (MRSA) is an important resistant phenotype of *S. aureus* which has acquired resistance through PBP2a (penicillin binding protein 2a) through blocking the proteins responsible for the construction and maintenance of the bacterial cell wall. The PBP2a protein is encoded by the *mecA* gene, which is the hallmark of MRSA. As opposed to the penicillinase gene, *mecA* does not reside on plasmids but on the chromosome, embedded in a large mobile genetic element called *staphylococcal chromosome cassette mec* or *SCCmec*. The presence of PBP2a means MRSA is not only resistant to methicillin, but also to all β-lactam antibiotics, including synthetic penicillins, cephalosporins and carbapenems (PANTOSTI and VENDITTI, 2009).

Vancomycin was identified as the first line of drug for treatment of MRSA, thus unfortunately use of vancomycin dramatically increased, not only for *S. aureus* infections but also for other infections, which resulted in the emergence of vancomycin resistance among bacterial populations (LOOMBA et al., 2010). Establishment of MRSA and the emergence of VRSA are of great concern because these are not only resistant to methicillin, but also to vancomycin, monobactams and cephalosporins, through the production of ESBL (Extended-spectrum beta-lactamases). Antibiotic resistance, the overuse of antibiotics, increased healthcare costs and sepsis-related deaths give rise to the need for exploration of the phenotypic and genotypic variations of resistance among *S. aureus* strains obtained from human and animal infections.

Considering the importance of *S. aureus* as the cause of various ailments in humans and animals, and variations in the strains in relation to antimicrobial resistance, the present study was undertaken with the objective of discovering the antibiotic resistance patterns of *S. aureus* from clinical and non-clinical sources of human and animal origin, and their statistical analysis, to ascertain the variable associations between isolates, according to their source of origin.

Material and methods

Bacterial strain. In the present investigation, 157 genotypically confirmed (species specific 23S rRNA sequence based based) *S. aureus* isolates were examined, of which 35 isolates were obtained from clinical infections of humans, 20 from pieces of meat from butcher's shops, three from horse skin wounds, two from pig nasal cavities, eight from camel skin wounds, six from dog skin infections, six from clinical infections of sheep, 21 from mastitic buffalo milk, 28 from mastitic goats' milk and 28 isolates were obtained from mastitic cows' milk samples.

Antibiotics susceptibility testing. The sensitivity test was conducted using the method of BAUER et al. (1966), against 42 antibiotics of different generations (Table 1), belonging to various groups.

The isolates were inoculated into sterile 5 mL Mueller-Hinton Broth tubes, and incubated for 18 hours at 37 °C. The opacity was adjusted to 0.5 McFarland opacity standard, and inoculums were well spread over the Mueller-Hinton agar surface, with the help of a sterilized spreader. The plates were allowed to dry for 10 minutes at 37 °C, and then antibiotic discs (Table 1, Hi Media, Mumbai) were carefully placed on the surface with enough space around each disc for diffusion of the antibiotic. The plates were incubated for 24 hours at 37 °C, and the diameter of the zone of inhibition of growth around each disc was measured in millimeters. After measurement of the inhibition zone, interpretation of resistant, sensitive and intermediates was made using the breakpoints defined by The Clinical & Laboratory Standards Institute (CLSI, 2016).

Multiple antibiotic resistance index (MAR) value. All multidrug resistant isolates were evaluated for their Multiple Antibiotic Resistance (MAR) index. In assessment of MDR isolates, this index was calculated as per the method given by KRUMPERMAN (1983).

MAR Index of single isolate = a/b, where *a* represents the number of antibiotics to which the isolate was resistant and *b* represents the number of antibiotics to which the isolate was exposed.

*Group MAR index value = a / (b*c)*, where *a* is the aggregate antibiotic resistance score of all isolates in the group, *b* is the number of antibiotics to which the isolate was exposed, and *c* is the total number of isolates in the group

Statistical analysis. For comparative analysis and to find variability between the antibiotic resistance patterns of *S. aureus* isolates of different origins, the diameter of the zone of inhibition of various antibiotics was recorded separately for each isolate. Then the mean diameter of the zone of inhibition of the isolates (ANOVA), the Duncan multiple range test (DMRT) analysis, Bonferroni correction and frequency of resistant, sensitive and intermediates were determined according to the method of CAMPBELL et al. (2007), using Microsoft excel and IBM SPSS Advanced Statistics 20.0 version software.

Results

In the present investigation, 157 *Staphylococcus aureus* isolates obtained from animals (cattle, buffalo, goat, sheep, dog, camel, pig and horse), humans and pieces of meat from local butcher's shops were subjected to detection and analysis of antibiotic resistance patterns against 42 antibiotics belonging to different categories and generations. The isolates exhibited significant variations in sensitivity patterns (Table 1). Overall, it was recorded that all (100%) isolates were sensitive to meropenem, chloramphenicol (except one human isolate, H28), and nitrofurantoin (except one cattle isolate, C39), more than 85% isolates were sensitive to nine antibiotics, namely: ampicillin+sulbactum (96.2%), imipenem (95.5%), cefalothin (91.7%), piperacillin+ tazobactam (91.7%), tobramycin (89.8%), doxycycline hydrochlorid (89.2%), ceftazidime + clavulanic acid (88.5%), oxacillin (87.3%) and polymxin-B (86.0%). More than 95.0% isolates were resistant to ampicillin and penicillin-G. Statistical analysis revealed that the Mean Sum of Square (MSS) of the diameter of the inhibition zone (mm) of each antibiotic showed highly significant ($P \leq 0.01$) variations between isolates from different sources in their resistance patterns for 39 antibiotics, significant variations ($P \leq 0.05$) for levofloxacin and nitrofurantoin, and a non-significant variation ($P > 0.05$) for clindamycin (Table 2).

In the present study, highly significant ($P \leq 0.01$) variations were recorded between *S. aureus* isolates for most of the antibiotics, thus all sources of isolates were further subjected to Duncan's Homogeneous Subsets analysis (DMRT) of Mean ± SEM values of the diameter of inhibition zone of the antibiotics. In the analysis, a maximum of five subsets were found for five antibiotics, namely: cefaclor, cefixime, cefixime+ clavulanic acid, imipenem and ticarcillin, and four subsets were found for 13 antibiotics, namely: ampicillin, ampicillin+ sulbactum, cefoparazone, ceftazidime+clavulanic acid, doxycycline hydrochloride, linezolid, nitrofurantoin, norfloxacin, penicillin-G, piperacillin, piperacillin + tazobactam, tetracycline and ticarcillin + clavulanic acid among *S. aureus* isolates. For the other antibiotics, two or three subsets were found among *S. aureus* isolates.

Table 1. Antibiotic Sensitivity patterns for *Staphylococcus aureus* isolates from various sources

S. N°	Name of Antibiotic	Antibiogram pattern of *S. aureus* isolates (%)																	
		Human			Meat piece			Horse			Pig			Camel			Dog		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
1.	Ampicillin	35 (100.0)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)
2.	Ampicillin+ Sulbactum	3 (8.6)	3 (8.6)	29 (82.8)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)
3.	Azithromycin	33 (94.3)	0 (0.0)	2 (5.7)	20 (100.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	2 (100)	7 (87.5)	0 (0.0)	1 (12.5)	2 (33.3)	0 (0.0)	4 (66.7)
4.	Aztreonam	17 (48.6)	9 (25.7)	9 (25.7)	0 (0.0)	4 (20.0)	16 (80.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	2 (25.0)	2 (25.0)	4 (50.0)	0 (0.0)	0 (0.0)	6 (100.0)
5.	Cefaclor	23 (65.7)	5 (14.3)	7 (20.0)	9 (45.0)	4 (20.0)	7 (35.0)	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	2 (100.0)	4 (50.0)	2 (25.0)	2 (25.0)	0 (0.0)	0 (0.0)	6 (100.0)
6.	Cefalothin	9 (25.7)	1 (2.9)	25 (71.4)	0 (0.0)	2 (10.0)	18 (90.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)
7.	Cefepime	11 (31.4)	9 (25.7)	15 (42.9)	0 (0.0)	6 (30.0)	14 (70.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	1 (12.5)	1 (12.5)	6 (75.0)	0 (0.0)	0 (0.0)	6 (100.0)
8.	Cefixime	30 (85.7)	2 (5.7)	3 (8.6)	11 (55.0)	1 (5.0)	8 (40.0)	3 (100.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	2 (33.3)	3 (50.0)	1 (16.7)
9.	Cefixime + Clavulanic acid	27 (77.1)	2 (5.7)	6 (17.2)	7 (35.0)	6 (30.0)	7 (35.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	3 (37.5)	3 (37.5)	2 (25.0)	0 (0.0)	2 (33.3)	4 (66.7)
10.	Cefoparazone	14 (40.0)	18 (51.4)	3 (8.6)	10 (50.0)	7 (35.0)	3 (15.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	2 (100.0)	0 (0.0)	2 (25.0)	4 (50.0)	2 (25.0)	0 (0.0)	3 (50.0)	3 (50.0)
11.	Cefoparazone + Sulbactam	6 (17.1)	19 (54.3)	10 (28.6)	0 (0.0)	6 (30.0)	14 (70.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	3 (37.5)	5 (62.4)	0 (0.0)	0 (0.0)	6 (100.0)
12.	Cefotaxime	11 (31.4)	15 (42.9)	9 (25.7)	1 (5.0)	6 (30.0)	13 (65.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	1 (50.0)	1 (50.0)	1 (12.5)	4 (50.0)	3 (37.5)	0 (0.0)	0 (0.0)	6 (100.0)
13.	Cefotaxime+ Clavulanic acid	8 (22.9)	15 (42.9)	12 (34.3)	1 (5.0)	2 (10.0)	17 (85.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	3 (37.5)	5 (62.5)	0 (0.0)	0 (0.0)	6 (100.0)
14.	Cefoxitin	26 (31.4)	0 (0.0)	9 (25.7)	7 (35.0)	0 (0.0)	13 (65.0)	1 (33.3)	0 (0.0)	2 (66.7)	1 (50.0)	0 (0.0)	1 (50.0)	4 (50.0)	0 (0.0)	4 (50.0)	0 (0.0)	0 (0.0)	6 (100.0)
15.	Ceftazidime	21 (60.0)	6 (17.1)	8 (22.9)	4 (20.0)	9 (45.0)	7 (35.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	1 (50.0)	1 (50.0)	3 (37.5)	4 (50.0)	1 (12.5)	0 (0.0)	3 (50.0)	3 (50.0)
16.	Ceftazidime + Clavulanic acid	8 (22.9)	3 (8.6)	24 (68.6)	0 (0.0)	2 (10.0)	18 (90.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (12.5)	7 (87.5)	0 (0.0)	0 (0.0)	6 (100.0)

Table 1. Antibiotic Sensitivity patterns for *Staphylococcus aureus* isolates from various sources (continued)

S. N°	Name of Antibiotic	Antibiogram pattern of *S. aureus* isolates (%)																			
		Human			Meat piece			Horse			Pig			Camel			Dog				
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S		
17.	Ceftriaxone	8 (22.9)	18 (51.4)	9 (25.7)	0 (0.0)	5 (25.0)	15 (75.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	1 (12.5)	3 (37.5)	4 (50.0)	0 (0.0)	0 (0.0)	6 (100.0)		
18.	Cefzolin	12 (34.3)	4 (11.4)	19 (54.3)	2 (10.0)	4 (20.0)	14 (70.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	4 (50.0)	4 (50.0)	0 (0.0)	0 (0.0)	6 (100.0)		
19.	Chloramphenicol	1 (2.9)	0 (0.0)	34 (97.1)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)		
20.	Ciprofloxacin	19 (54.3)	15 (42.9)	1 (2.9)	6 (30.0)	13 (65.0)	1 (5.0)	0 (0.0)	3 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (12.5)	6 (75.0)	1 (12.5)	0 (0.0)	6 (100.0)	0 (0.0)		
21.	Clindamycin	2 (5.7)	13 (37.1)	20 (57.2)	0 (0.0)	4 (20.0)	16 (80.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	5 (62.5)	3 (37.5)	0 (0.0)	0 (0.0)	6 (100.0)		
22.	Cloxacillin	14 (40.0)	20 (57.2)	1 (2.9)	1 (5.0)	16 (80.0)	3 (15.0)	0 (0.0)	2 (66.7)	1 (33.3)	2 (100.0)	0 (0.0)	0 (0.0)	1 (12.5)	6 (75.0)	1 (12.5)	0 (0.0)	0 (0.0)	6 (100.0)		
23.	Doxycycline Hydrochloride	3 (8.6)	5 (14.3)	27 (77.1)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (12.5)	7 (87.5)	0 (0.0)	0 (0.0)	6 (100.0)		
24.	Faropenem	17 (48.6)	14 (40.0)	4 (11.4)	5 (25.0)	12 (60.0)	3 (15.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	1 (50.0)	1 (50.0)	1 (12.5)	5 (62.5)	2 (25.0)	0 (0.0)	0 (0.0)	6 (100.0)		
25.	Gentamicin	8 (22.9)	2 (5.7)	25 (71.4)	3 (15.0)	0 (0.0)	17 (85.0)	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	1 (50.0)	1 (50.0)	5 (62.5)	2 (25.0)	1 (12.5)	0 (0.0)	0 (0.0)	6 (100.0)		
26.	Imipenem	5 (14.3)	2 (5.7)	28 (80.0)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)		
27.	Levofloxacin	0 (0.0)	5 (14.3)	30 (85.7)	1 (5.0)	5 (25.0)	14 (70.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	3 (37.5)	5 (62.5)	0 (0.0)	0 (0.0)	6 (100.0)		
28.	Linezolid	3 (8.6)	0 (0.0)	32 (91.4)	1 (5.0)	0 (0.0)	19 (95.0)	0 (0.0)	0 (0.0)	3 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	5 (62.5)	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)	6 (100.0)		
29.	Meropenem	0 (0.0)	0 (0.0)	35 (100.0)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)		
30.	Methicillin	10 (28.6)	15 (42.9)	10 (28.6)	2 (10.0)	11 (55.0)	7 (35.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	1 (12.5)	2 (25.0)	5 (62.5)	0 (0.0)	1 (16.7)	5 (83.3)		
31.	Nitrofurantoin	0 (0.0)	1 (2.9)	34 (97.1)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)		
32.	Norfloxacin	29 (89.9)	5 (14.3)	1 (2.9)	16 (80.0)	3 (15.0)	1 (5.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	1(12.5)	7 (87.5)	0 (0.0)	0 (0.0)	4 (66.7)	2 (33.3)		

Table 1. Antibiotic Sensitivity patterns for *Staphylococcus aureus* isolates from various sources (continued)

S. No	Name of Antibiotic	Antibiogram pattern of *S. aureus* isolates (%)																	
		Human			Meat piece			Horse			Pig			Camel			Dog		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
33.	Oxacillin	13 (37.1)	1 (2.9)	21 (60.0)	2 (10.0)	0 (0.0)	18 (90.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)
34.	Penicillin -G	35 (100)	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)
35.	Piperacillin	34 (97.1)	1 (2.9)	0 (0.0)	20 (100.0)	0 (0.0)	0 (0.0)	3	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)
36.	Piperacillin+ Tazobactam	11 (31.4)	0 (0.0)	24 (68.6)	2 (10.0)	0 (0.0)	18 (90.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)
37.	Polymxin -B	5 (14.3)	0 (0.0)	30 (85.7)	4 (20.0)	0 (0.0)	16 (80.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	3 (37.5)	0 (0.0)	5 (62.5)	1 (16.7)	0 (0.0)	5 (83.3)
38.	Tetracycline	6 (17.1)	15 (42.9)	14 (40.0)	0 (0.0)	1 (5.0)	19 (95.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	3 (37.5)	1 (12.5)	4 (50.0)	0 (0.0)	0 (0.0)	6 (100.0)
39.	Ticarcillin	7 (20.0)	16 (45.7)	12 (34.3)	0 (0.0)	3 (15.0)	17 (85.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (12.5)	7 (87.5)	0 (0.0)	0 (0.0)	6 (100.0)
40.	Ticarcillin+ Clavulanic Acid	28 (80.0)	0 (0.0)	7 (20.0)	12 (60.0)	0 (0.0)	8 (40.0)	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	3 (37.5)	0 (0.0)	5 (62.5)	0 (0.0)	0 (0.0)	6 (100.0)
41.	Tobramycin	15 (42.9)	0 (0.0)	20 (57.2)	0 (0.0)	0 (0.0)	20 (100.0)	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)
42.	Vancomycin	18 (51.4)	16 (45.7)	1 (2.9)	5 (25.0)	13 (65.0)	2 (10.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	7 (87.5)	1 (12.5)	0 (0.0)	4 (66.7)	2 (33.3)

S. No.	Name of Antibiotic	Antibiogram pattern of *S. aureus* isolates (%)														
		Sheep			Buffalo			Goat			Cattle			Total		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
1.	Ampicillin	6 (100.0)	0 (0.0)	0 (0.0)	21 (100)	0 (0.0)	0 (0.0)	27 (96.4)	0 (0.0)	1 (3.5)	25 (89.3)	0 (0.0)	3 (10.7)	153 (97.5)[a]	0 (0.0)	4 (2.5)
2.	Ampicillin+ Sulbactum	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	3 (1.9)	3 (1.9)	151 (96.2)[c]
3.	Azithromycin	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	4 (21.0)	17 (80.0)	3 (10.7)	4 (14.3)	21 (75.0)	10 (35.7)	5 (17.9)	13 (46.4)	76 (48.4)	14 (8.9)	67 (42.7)

Table 1. Antibiotic Sensitivity patterns for *Staphylococcus aureus* isolates from various sources (continued)

S. No.	Name of Antibiotic	Antibiogram pattern of *S. aureus* isolates (%)														
		Sheep			Buffalo			Goat			Cattle			Total		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
4.	Aztreonam	0 (0.0)	1 (16.7)	5 (83.3)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	3 (10.7)	25 (89.3)	4 (14.3)	6 (21.4)	18 (64.3)	24 (15.3)	28 (17.8)	105 (66.9)
5.	Cefaclor	0 (0.0)	0 (0.0)	6 (100.0)	1 (4.8)	3 (14.3)	17 (81.0)	1 (3.6)	4 (14.3)	23 (82.1)	5 (17.9)	6 (21.4)	17 (60.7)	45 (28.6)	24 (15.3)	88 (56.1)
6.	Cefalothin	0 (0.0)	0 (0.0)	6 (100.0)	1 (4.8)	0 (0.0)	20 (95.2)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	10 (6.4)	3 (1.9)	144 (91.7)[c]
7.	Cefepime	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	1 (3.6)	6 (21.4)	21 (75.0)	13 (8.3)	23 (14.6)	121 (77.1)
8.	Cefixime	0 (0.0)	1 (16.7)	5 (83.3)	6 (28.6)	12 (57.1)	3 (14.3)	1 (3.6)	13 (46.4)	14 (50.0)	14 (50.0)	6 (21.4)	8 (28.6)	76 (48.4)	39 (24.8)	42 (26.8)
9.	Cefixime + Clavulanic acid	0 (0.0)	0 (0.0)	6 (100.0)	4 (19.0)	7 (33.3)	10 (47.6)	0 (0.0)	2 (7.1)	26 (92.9)	7 (25.0)	6 (21.4)	15 (53.6)	48 (30.6)	28 (17.8)	81 (51.6)
10.	Cefoparazone	0 (0.0)	1 (16.7)	5 (83.3)	1 (4.8)	12 (57.1)	8 (38.1)	1 (3.6)	11 (39.3)	16 (57.1)	3 (10.7)	9 (32.1)	16 (57.1)	31 (19.7)	69 (43.9)	57 (36.3)
11.	Cefoparazone + Sulbactam	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	1 (4.8)	20 (95.2)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	9 (32.1)	19 (67.1)	6 (3.8)[d]	39 (24.8)	112 (71.3)
12.	Cefotaxime	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	4 (19.0)	17 (81.0)	0 (0.0)	2 (7.1)	26 (92.9)	0 (0.0)	10 (35.1)	18 (64.3)	13 (8.3)	43 (27.4)	101 (64.1)
13.	Cefotaxime+ Clavulanic acid	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	6 (21.4)	22 (78.6)	9 (5.7)[d]	27 (17.2)	121 (77.1)
14.	Cefoxitin	0 (0.0)	0 (0.0)	6 (100.0)	2 (9.5)	0 (0.0)	19 (90.5)	2 (7.1)	0 (0.0)	26 (92.9)	12 (42.9)	0 (0.0)	16 (57.1)	55 (5.0)	0 (0.0)	102 (65.0)
15.	Ceftazidime	0 (0.0)	0 (0.0)	6 (100.0)	1 (4.8)	4 (19.0)	16 (76.2)	1 (3.6)	5 (17.9)	22 (78.6)	6 (21.4)	8 (28.6)	14 (50.0)	37 (23.6)	41 (26.1)	79 (50.3)
16.	Ceftazidime + Clavulanic acid	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	3 (7.1)	25 (89.3)	8 (5.1)	10 (6.4)	139 (88.5)[c]
17.	Ceftrioxane	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	2 (9.5)	19 (90.5)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	7 (25.0)	21 (75.0)	9 (5.7)[d]	36 (22.9)	112 (71.3)
18.	Cefzolin	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21(100.0)	0 (0.0)	1 (3.6)	27	0 (0.0)	1 (3.6)	27 (94.4)	14 (8.9)	15 (9.6)	128 (81.5)
19.	Chloramphenicol	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	1 (0.6)	0 (0.0)	156 (99.5)[b]

Table 1. Antibiotic Sensitivity patterns for *Staphylococcus aureus* isolates from various sources (continued)

S. No.	Name of Antibiotic	Antibiogram pattern of *S. aureus* isolates (%)															
		Sheep			Buffalo			Goat			Cattle			Total			
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	
20.	Ciprofloxacin	0 (0.0)	5 (83.3)	1 (16.7)	1 (4.8)	16 (76.2)	4 (19.0)	3 (10.7)	18 (64.3)	7 (25.0)	3 (10.7)	11 (39.3)	14 (50.0)	34 (21.7)	94 (59.9)	29 (18.5)	
21.	Clindamycin	0 (0.0)	2 (33.3)	4 (66.7)	0 (0.0)	8 (38.1)	13 (61.9)	0 (0.0)	10 (35.7)	18 (64.3)	1 (3.6)	10 (35.1)	17 (60.7)	3 (1.9)[d]	54 (34.4)	100 (63.7)	
22.	Cloxacillin	0 (0.0)	5 (83.3)	1 (16.7)	2 (9.5)	8 (38.1)	11 (52.4)	0 (0.0)	14 (50.0)	14 (50.0)	4 (14.3)	18 (64.3)	6 (21.4)	24 (15.3)	89 (56.7)	44 (28.0)	
23.	Doxycyline Hydrochlorid	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	1 (3.6)	27 (94.4)	5 (17.9)	2 (7.1)	21 (75.0)	8 (5.1)	9 (5.7)	140 (89.2)[e]	
24.	Faropenem	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	4 (19.0)	17 (81.0)	0 (0.0)	3 (10.7)	25 (89.3)	2 (7.1)	9 (32.1)	17 (60.7)	25 (15.9)	50 (31.8)	82 (52.2)	
25.	Gentamicin	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	2 (9.5)	19 (90.5)	0 (0.0)	0 (0.0)	28 (100.0)	4 (14.3)	5 (17.9)	19 (67.9)	21 (13.4)	12 (7.6)	124 (79.0)	
26.	Imipenem	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	5 (3.2)	2 (1.3)	150 (95.5)[e]	
27.	Levofloxacin	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	4 (19.0)	17 (81.0)	0 (0.0)	2 (7.1)	26 (92.9)	2 (7.1)	3 (10.7)	23 (82.1)	3 (1.9)[d]	23 (14.6)	131 (83.4)	
28.	Linezolid	0 (0.0)	0 (0.0)	6 (100.0)	1 (4.8)	0 (0.0)	20 (95.2)	3 (10.7)	0 (0.0)	25 (89.3)	9 (32.1)	0 (0.0)	19 (67.9)	24 (15.3)	0 (0.0)	133 (84.7)	
29.	Meropenem	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	157 (100.0)[b]	
30.	Methicillin	0 (0.0)	0 (0.0)	6 (100.0)	1 (4.8)	7 (33.3)	13 (61.9)	0 (0.0)	2 (7.1)	26 (92.9)	5 (17.9)	5 (17.9)	18 (64.3)	19 (12.1)	44 (28.0)	94 (59.9)	
31.	Nitrofurantoin	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21(100.0)	0 (0.0)	0 (0.0)	28 (100.0)	1 (3.6)	0 (0.0)	27 (96.4)	1 (0.6)	1 (0.6)	155 (98.7)[b]	
32.	Norfloxacin	0 (0.0)	3 (50.0)	3 (50.0)	2 (9.5)	9 (42.9)	10 ()	5 (17.9)	9 (32.1)	14 (50.0)	5 (17.9)	6 (21.4)	17 (60.7)	63 (40.1)	46 (29.3)	48 (30.6)	
33.	Oxacillin	0 (0.0)	0 (0.0)	6 (100.0)	2 (9.5)	0 (0.0)	19 (90.5)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	2 (7.1)	26 (92.9)	17 (10.8)	3 (1.9)	137 (87.3)[e]	

Table 1. Antibiotic Sensitivity patterns for *Staphylococcus aureus* isolates from various sources (continued)

S. No.	Name of Antibiotic	Antibiogram pattern of *S. aureus* isolates (%)														
		Sheep			Buffalo			Goat			Cattle			Total		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
34.	Penicillin -G	5 (83.3.0)	0 (0.0)	1 (1.6)	20 (95.2)	0 (0.0)	1 (4.8)	28 (100.0)	0 (0.0)	0 (0.0)	27	0 (0.0)	1 (3.6)	155 (98.1)[a]	0 (0.0)	3 (1.9)
35.	Piperacillin	1 (16.7)	0 (0.0)	5 (83.3)	16 (76.1)	1 (4.8)	4 (19.0)	11 (39.3)	9 (32.1)	8 (28.6)	14 (50.0)	3 (10.7)	11 (39.3)	115 (73.2)	14 (8.9)	28 (17.8)
36.	Piperacillin+ Tazobactam	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	13 (8.3)	0 (0.0)	144 (91.7)[c]
37.	Polymxin -B	1 (16.7)	0 (0.0)	5 (83.3)	2 (9.5)	0 (0.0)	19 (90.5)	6 (21.4)	0 (0.0)	22 (78.6)	0 (0.0)	0 (0.0)	28 (100.0)	22 (14.0)	0 (0.0)	135 (86.0)[c]
38.	Tetracycline	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	1 (4.8)	20 (95.2)	0 (0.0)	2 (7.1)	26 (78.6)	8 (28.6)	4 (14.3)	16 (57.1)	17 (10.8)	24 (15.3)	116 (73.9)
39.	Ticarcillin	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	1 (3.6)	3 (10.7)	24 (85.7)	8 (5.1)[d]	25 (15.9)	124 (79.0)
40.	Ticarcillin + Clavulanic Acid	0 (0.0)	0 (0.0)	6 (100.0)	3 (14.3)	0 (0.0)	18 (85.7)	2 (7.1)	0 (0.0)	26 (92.9)	6 (21.4)	0 (0.0)	22 (78.6)	55 (35.0)	0 (0.0)	102 (65.0)
41.	Tobramycin	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	21 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	28 (100.0)	16 (10.1)	0 (0.0)	141 (89.8)[c]
42.	Vancomycin	6 (100.0)	0 (0.0)	0 (0.0)	12 (57.1)	9 (42.9)	0 (0.0)	6 (21.4)	22 (78.6)	0 (0.0)	3 (10.7)	23 (82.1)	2 (7.1)	50 (31.8)	97 (61.8)	10 (6.4)

Abbreviations: R- Resistant, I- Intermediate, S- Sensitive. Superscript:- a- More than 95.0% isolates were resistant for Ampicillin (One isolate from Goat- AG17 and three isolates from cattle-C3R, C9 and C17 were sensitive) and Penicillin -G (one isolate from sheep- SV4, one from buffalo-B36 and one isolate from cattle C3R were sensitive), b- 100 % isolates were sensitive for Chloramphenicol (except one Human isolate (H28) was resistant), Meropenem and Nitrofurantoin (except one cattle isolate (C39) was resistant), c- More than 85% isolates were sensitive for Ampicillin+ Sulbactum (only three isolates from human (H2, H3 and H4) were resistant), Cefalothin, Ceftazidime+ Clavulanic Acid, Doxycyline Hydrochlorid, Imipenem, Oxacillin, Piperacillin+ Tazobactam, Polymxin -B and Tobramycin, d- Isolate which having more no. of intermediates and sensitives but less no. of resistant for Cefoparazone+ Sulbactam, Cefotaxime+ Clavulanic Acid, Ceftrioxane, Clindamycin, Levofloxacin and Ticarcillin

Table. 2. Analysis of variance of diameter of inhibition zone (mm) of antibiotics for *S. aureus* isolates

S. No.	Source of variation/Antibiotic	MSS
1.	Ampicillin	288.043**
2.	Ampicillin+ Sulbactum	271.987**
3.	Azithromycin	789.142**
4.	Aztreonam	157.118**
5.	Cefaclor	429.189**
6.	Cefalothin	353.234**
7.	Cefepime	180.461**
8.	Cefixime	411.040**
9.	Cefixime+ Clavulanic Acid	298.578**
10.	Cefoparazone	152.219**
11.	Cefoparazone+ Sulbactam	165.105**
12.	Cefotaxime	268.889**
13.	Cefotaxime+ Clavulanic Acid	207.341**
14.	Cefoxitin	304.429**
15.	Ceftazidime	134.165**
16.	Ceftazidime+ Clavulanic Acid	163.499**
17.	Ceftrioxane	302.930**
18.	Cefzolin	349.944**
19.	Chloramphenicol	25.004**
20.	Ciprofloxacin	64.327**
21.	Clindamycin	9.628^{NS}
22.	Cloxacillin	476.205**
23.	Doxycycline Hydrochloride	141.901**
24.	Faropenem	536.097**
25.	Gentamicin	50.272**
26.	Imipenem	937.524**
27.	Levofloxacin	17.739*
28.	Linezolid	41.257**
29.	Meropenem	121.492**
30.	Methicillin	160.922**
31.	Nitrofurantoin	11.777*
32.	Norfloxacin	194.456**
33.	Oxacillin	351.371**
34.	Penicillin -G	256.907**
35.	Piperacillin	399.747**
36.	Piperacillin+ Tazobactam	149.886**
37.	Polymxin -B	24.501**
38.	Tetracycline	197.135**
39.	Ticarcillin	322.626**
40.	Ticarcillin+ Clavulanic Acid	350.513**
41.	Tobramycin	77.792**
42.	Vancomycin	10.179**

i. - Degree of freedom (*df*) = 9; ii. - * = Significant (P≤0.05); iii. - ** Highly significant (P≤0.01); iv. - NS = Non-significant (P>0.05); MSS = Mean Sum of Square;

Table 3. Detection of group Multiple Antibiotic Resistance Index (MAR) value among sources of *S. aureus* isolate

S. No.	Source of Isolate	Total No. of isolate	Aggregate antibiotic resistance score	Group MAR index value	Significance
1.	Human	35	585	0.40	Greater than 0.2 MAR Index of group indicates that several antibiotics were used in that group and more than 0.2 MAR group is an high risk potential source of spread MDR
2.	Meat piece	20	192	0.24	
3.	Horse	3	27	0.21	
4.	Pig	2	19	0.23	
5.	Camel	8	84	0.25	
6.	Dog	6	23	0.10	
7.	Sheep	6	19	0.08	
8.	Buffalo	21	89	0.10	
9.	Goat	28	100	0.09	
10.	Cattle	28	187	0.16	

Decreasing Order of MAR index Value: - Human > Camel > Meat pieces > Pig > Horse > Cattle > Dog = Buffalo > Goat > Sheep

Table 4. Detection and Distribution of Multiple Antibiotic Resistance Index (MAR) value among individual *S. aureus* isolates

S. No.	MAR Index Value Type (MAR)	Isolate I.D.	No. of Isolate	No. of antibiotic, which the isolate was resistant	MAR Index Value	Significance
1.	MAR1	H2	1	34	0.81	66 (42%) isolates had 0.2 or more than 0.2 MAR index value with high risk potential source of spread MDR
2.	MAR2	H1 and H8	2	32	0.76	
3.	MAR3	H3 and H4	2	31	0.74	
4.	MAR4	H5	1	29	0.69	
5.	MAR5	H29	1	28	0.67	
6.	MAR6	H6	1	25	0.6	
7.	MAR7	H27	1	23	0.55	
8.	MAR8	J4	1	21	0.50	
9.	MAR9	H25, Mt26	2	20	0.48	
10	MAR10	H7 and H48	2	19	0.45	
11.	MAR11	H24, H39, H46 and C29	4	18	0.43	
12.	MAR12	H40	1	17	0.40	
13	MAR13	H30 and Mt2	2	16	0.38	
14.	MAR14	H28, J14 and C39	3	15	0.36	
15.	MAR15	H33, H37, H45, Mt3, C37 and C43	6	14	0.33	
16.	MAR16	Mt4, Mt9 and J18	3	13	0.31	
17.	MAR17	Hrs3 and C12	2	12	0.29	
18.	MAR18	H12, H21, H34, H44, Mt27, Pg2 and C52	7	11	0.26	
19.	MAR19	H14, H22, Mt19, J3, B24, C13, C41 and C46	8	10	0.24	
20.	MAR20	H11, H15, Mt13, C34 and Hrs4	5	9	0.21	
21.	MAR21	H41, Mt1, Mt10, Mt15, Mt22, Mt24, Mt25, Mt28, Pg4, B27 and C5R	11	8	0.20	

Table 4. Detection and Distribution of Multiple Antibiotic Resistance Index (MAR) value among individual *S. aureus* isolates (continued)

S. No.	MAR Index Value Type (MAR)	Isolate I.D.	No. of Isolate	No. of antibiotic, which the isolate was resistant	MAR Index Value	Significance
22.	MAR22	H13, Mt14, Mt20, Hrs1, J9, J10, B1, B46, B55, C36 and C40	11	7	0.17	91 (58%) isolates had less than 0.2 MAR index value with less risk source of MDR
23.	MAR23	H10, Mt11, Mt12, J15, AG8 and G9	6	6	0.14	
24.	MAR24	H9, H16, H31, J2, D7, D9, SN4, B23, B26, B30, B39, AG15, G24, G29, G39, G46, G49, C23 and C26	19	5	0.12	
25.	MAR25	Mt31, D4, B21, B28, B31, B34, B42, B43, B57, AG13, G1, G7, G16 and G21	14	4	0.10	
26.	MAR26	D6, D10, D13, SV2, SV3, SN3, SN14, B29, B36, AG5, AG6, AG17, G2, G11, G40, G41, G55, C2R, C7, C8, C11, C17, C20 and C50	24	3	0.07	
27.	MAR27	SV4, B5, B10, B19, AG10, G10, G35, G37, G43, G45, G47, C2, C9, C15, C22 and C47	16	2	0.05	
28.	MAR28	C3R	1	1	0.02	

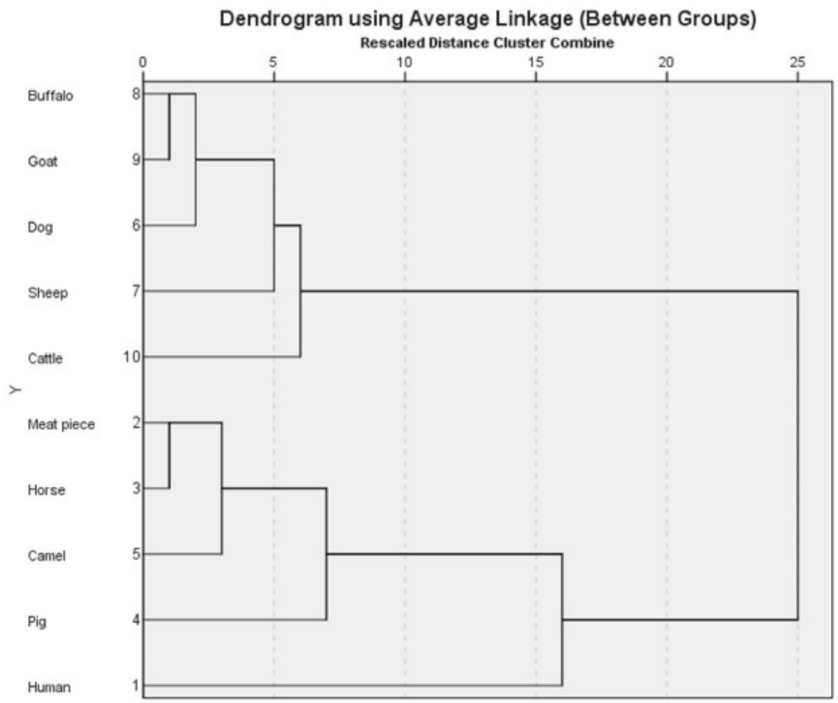

Fig. 1. Hierarchical ascendant cluster analysis of antibiotic sensitivity pattern through mean values of the diameter of the inhibition zone (mm) of antibiotics against *S. aureus* isolates

Further, the Bonferroni correction was carried out to find the exact value of the probability level of variance among sources of *S. aureus* isolates belonging to different Duncan's Homogeneous Subsets. It was found that isolates from human sources were significantly variable, with P≤0.0001 probability level of variance, in relation to other pieces of meat and animal origin sources, for most of antibiotics. Isolates from horse, camel and pig sources were non-significantly variable with each other for most antibiotics and the sheep, goat, buffalo and cattle isolates were non-significantly variable with each other for most of the antibiotics.

Hierarchical ascendant cluster analysis of antibiotic sensitivity patterns, through the mean values of the diameter of the inhibition zone (mm) of antibiotics against *S. aureus* isolates from each group of origin, was carried out using Squared Euclidean Distance (SED) and the between-groups linkage methods (Fig. 1). The sources of isolates were clustered into three groups at 10.0 rescaled cluster distance, one cluster comprising buffalo, cattle, sheep, dog and goat sources isolates, the second cluster included horse, camel, meat piece and pig isolates, while the third cluster included human sources isolates (Fig. 1). All three clusters had significant variation (P≤0.05) between them. The first cluster of buffalo, cattle, sheep, dog and goat sources exhibited the lowest resistance and the third cluster of human isolates showed the highest resistance for most of the antibiotics (Table 1). An overall lower resistance was recorded among animal origin isolates in comparison to human origin isolates.

In the present investigation, all the *S. aureus* isolates (eExcept one cattle/C3R) were multidrug resistant (MDR) isolates. In risk assessment of MDR isolates, all multidrug resistant isolates were evaluated for both their group and individual Multiple Antibiotic Resistance (MAR) index (KRUMPERMAN, 1983). In the MAR group, *S. aureus* isolates from human sources (0.40) had the highest MAR index, and sources from camels (0.25), meat pieces (0.24), pigs (0.23) and horses (0.21) had more than 0.20 MAR in decreasing order. Other groups of *S. aureus* isolates of animal sources, such as cattle, dogs, buffalo, goats and sheep had less than 0.20 MAR, as described in Table 3. In the individual isolate MAR index, with a total of 157 isolates, 66 (42%) isolates had a MAR index value of 0.2 or more than 0.2, with a high risk of being a potential source of MDR spread. These isolates comprised most of the human and meat isolates, while 91 (58%) isolates had a MAR index value less than 0.2 with less risk of being a source of MDR, and this included most of the animal origin isolates (Table 4).

Discussion

S. aureus is able to induce structural changes in the host and keeps on developing resistance against the most commonly used antibiotics. Over the last few decades, there has been a sudden increase in the use of antibiotics in veterinary as well as medical sciences, not only to control disease but also as a prophylactic measure to prevent bacterial infections secondary to viral infections. These resistant microorganisms become part of the environment and are transmitted from animals to humans, and *vice versa* (MEHNDIRATTA et al., 2009). Thus, the present study was designed to find variations and associations between different sources in regards to antibiotic resistance patterns, where we observed high resistance, higher MAR values and significant differences in resistance patterns between different sources.

As in the present study, we observed higher resistance for β-lactam antibiotics, GULER et al. (2005) also recorded highest resistance against β-lactam antibiotics, penicillin and ampicillin for *S. aureus* isolates from bovine clinical mastitic milk isolates. Similarly, TURUTOGLU et al. (2006), EBRAHIMI et al. (2007), PEREIRA et al. (2009) and HUSSAIN et al. (2012) also recorded maximum resistance for beta-lactam antibiotics against *S. aureus* isolates from various sources.

MOHANASOUNDARAM and LALITHA (2008) studied 150 isolates of *S. aureus* from human clinical infections, and similar results were reported for norfloxacin (100% resistance) and chloramphenicol (18% resistance). However, they also reported higher resistance towards tetracycline (82%), gentamicin (88%) and ciprofloxacin (97%). Similar to the present study, a non-significant

difference was observed in antibiotic sensitivity or resistance patterns against isolates from cattle and goats in the work by UPADHYAY and KATARIA (2009). Almost 80-90% of the milk isolates showed multiple drug resistance to the majority of the antimicrobial agents tested, such as: ampicillin, cloxacillin, kanamycin and vancomycin, while several isolates were found susceptible to tetracycline, oxacillin and ciprofloxacin (SHARMA et al., 2011).

YADAV et al. (2015) reported a similar antibiogram in the same area of study, who studied 32 *S. aureus* isolates obtained from mastitis infections in cattle and buffalo using 33 different antibiotics. They reported doxycycline, gentamicin, methicillin and tobramycin to be more effective against all isolates, and maximum resistance was exhibited against polymxin-B and cefixime, similar to our study results. Similar to the present study, ADAMS et al. (2018) studied the epidemiological associations between *S. aureus* isolates in relation to animal breed, species of organism, sample source, and time period. They reported significant ($P<0.05$) associations between the odds of AMR and horse breed, species of organism and year. Similarly, significant ($P<0.05$) associations were identified between the odds of MDR and breed and age.

Similar to the present study ADEYEMI et al. (2015) reported more than 0.2 MAR index among *S. aureus* isolates obtained from diseased human individuals. Similarly, VIJAYALAKSHMI et al. (2013) screened 12 *S. aureus* isolates from human wound samples for nine different groups of antibiotics, and found that 100% isolates were multidrug resistant with an MAR index of more than 0.22. Close to our results, UDOBI et al. (2013) reported the MAR index of *S. aureus* isolates obtained from various clinical samples from human sources, and detected that 79.6%, 60.6%, and 76.5% of wound, skin, and bed isolates had an MAR index greater than 0.25%.

SHAMILA-SYUHADA et al. (2016) studied the antibiotics resistance among *S. aureus* isolates isolated from raw milk samples obtained from small scale dairy farms, and reported MAR indexes ranging from 0.08 to 0.67. ALI et al. (2015) studied *S. aureus* isolates from mastitic milk samples of buffalo in Egypt, and found that most of the isolates had an MAR index more than 0.28 in comparison to the present study, while only a few isolates had MAR lower than 0.2. This index is an epidemiological tool used for analysis of the risk to the environment through bacterial contamination, and it is also used to assess whether a group of isolates/an individual isolate originated from an environment where several antibiotics were used or not. The index of an isolated group of bacteria/ individual bacteria, if greater than 0.2, implies that the strains of those bacteria originated from an environment where several antibiotics were used, and the group with MAR higher than 0.2 is an high risk potential source of spread of MDR.

Presently, there is growing concern among scientists regarding the increasing resistance in pathogens. The concerns are multifaceted, *viz*. inaccurate diagnosis, defective dosage, indiscriminate use, development of new drugs etc. Indiscriminate antibiotic use in dairy and other animals, as well as humans, leads to treatment failure, escalated treatment costs and development of resistance to antimicrobials. Thus, the multiplicity of the causes and emergence of resistance due to indiscriminate and prolonged use of antibiotics in the absence of an antibiogram is a major hurdle in the physical, chemical and microbiological control of infections.

In the present investigation, the highly significant difference in the antibiogram patterns between different sources of *S. aureus* may indicate the pattern and frequency of use of various antibiotics in humans and animals. The initial generations of antibiotics showed lower efficacy than the newer generations of antibiotics. The analysis of the antibiograms revealed that the susceptibility and resistance shown by the isolates was dependent on the use of the antibiotics and the source of the sample, i.e. the lower the use, the greater the susceptibility of the isolates detected. There have been many studies looking at *S. aureus* of various origins regarding their antibiogram patterns, and they have found that *S. aureus* is endowed with the capability of developing resistance towards an antibiotic, even when isolates are exposed for short periods. Further, the present study suggests that not

only the phenotypic comparative characterization of *S. aureus* isolates from different sources but also the genotypic aspect should be explored, in relation to antibiotic resistance.

Statement of animal rights
In the present investigation no animal or human experiments, clinical trials or any invasive methods were used.

Conflict of interest
No conflict of interest exists among authors.

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SAŽETAK

S obzirom na važnost bakterije *Staphylococcus aureus* u smislu otpornosti na antibiotike, cilj je ovog istraživanja bio otkriti varijacije među njezinim izolatima s obzirom na rezistenciju prema brojnim lijekovima. Istraživanje je obuhvatilo ukupno 157 vrsno specifičnih 23S rRNA *S. aureus* izolata iz različitih kliničkih i nekliničkih izvora životinja (goveda, bivoli, koze, ovce, psi, deve, svinje i konji), ljudi i mesa iz mesnica. Zabilježeno je više od 95 % izolata otpornih na ampicilin i penicilin-G dok je gotovo 100 % izolata bilo osjetljivo na kloramfenikol, meropenem i nitrofurantoin. Izolati iz različitih izvora pokazali su znakovite varijacije ($P \leq 0,01$) u rezistenciji na 39 antibiotika, znakovite varijacije ($P \leq 0,05$) za levofloksacin i nitrofurantoin, no nije bilo znakoviith varijacija ($P > 0,05$) za klindamicin. Primjenom Bonferronijeve korekcije izolati iz ljudi bili su znakovito različiti ($P < 0,0001$) u odnosu na uzorke iz mesa i drugih izvora animalnog podrijetla za većinu antibiotika. Izolati podrijetlom od ljudi imali su najviši MAR indeks (0,40). Uočena je vrlo znakovita razlika u antibiogramima među različitim izvorima bakterije *S. aureus* što može uputiti na način i učestalost primjene različitih antibiotika u ljudi i životinja.

Klučne riječi: *Staphylococcus aureus;* rezistencija na antibiotike; životinje; ljudi; DMRT analiza