Indian J Dairy Sci 79(3): 224-231
https://doi.org/10.33785/IJDS.2026.v79i03.004

RESEARCH ARTICLE

AMR profile of methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive S. aureus (MSSA) isolated from milk samples

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Received: 25 December 2025 / Accepted: 20 January 2026 / Published online: 23 July 2026

Abstract: The present study was conducted to determine the antimicrobial resistance profile of methicillin-resistant S. aureus and methicillin-sensitive *S. aureus* in milk samples. Quarter foremilk samples were collected from organized dairy herds and confirmed *S. aureus* isolates were subjected to antimicrobial sensitivity testing (AST) by disk diffusion method. Out of total 194 Staphylococci spp. isolated from milk, 103 were confirmed as S. aureus by PCR amplification of *nuc* gene. The overall prevalence of *S. aureus* was 53.1% (103/194). 61 (59.22 %) *S. aureus* isolates were phenotypically identified as MRSA and 42 (40.77%) were MSSA. In MRSA and MSSA, highest resistance was observed for penicillin and lowest was observed for co-trimoxazole, enrofloxacin, gentamicin and tetracycline. Higher level of resistance was observed in MRSA than MSSA. Among MRSA, antibiotic resistance gene of highest prevalence was blaZ (39.34 %), followed by aacA-aphD (29.51 %), tetK (22.95 %), tetM (9.83 %) and ermA (6.55%). Similar prevalence of antibiotic resistance genes was found in MSSA. *VanA* gene was not found in our study.

Keywords: AST, MRSA, Milk sample, Mastitis, Resistance genes

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Introduction

Staphylococcus aureus (*S. aureus*) is a major cause of mastitis, resulting in significant economic losses in dairy herds and the dairy industry (Kossaibati, 1997). Antimicrobial resistance has been documented in *S. aureus* isolates in several studies conducted in various countries (De Oliveira et al. 2000). Methicillin-resistant *S. aureus* (MRSA) are *S. aureus* isolates that produce additional penicillin-binding proteins, PBP2a or PBP2, causing resistance to all currently available b-lactam antibiotics. The mecA gene produces the protein PBP2a. However, additional genes, which are present in susceptible isolates, can also influence how methicillin resistance is expressed in S. aureus, leading to resistance variability and making resistance detection difficult. The main surveillance tool used to evaluate resistance patterns of mastitis pathogens is agar disc diffusion (Kirby-Bauer method) but its interpretation is not always uniform between studies and lacks correlation with minimum inhibitory concentration (MIC) values determined by standard dilution or agar diffusion methods (Gentilini et al. 2002). Methicillin-resistant S. aureus (MRSA) mastitis is common in dairy cattle, with a 16.47% frequency (Shrivastava et al. 2017). The use of antimicrobials in veterinary medicine creates a selective pressure for the emergence of antimicrobial resistant bacteria which leads to treatment failures (Sivakumar et al. 2019). Keeping in mind these facts, the present study was performed to determine the antimicrobial resistance profile of methicillin-resistant *S. aureus* (MRSA) along with antibiotic resistance gene typing (Mahanti et al. 2020). This study provides a comparative molecular and phenotypic antimicrobial resistance profiling of MRSA and MSSA isolated from dairy milk, highlighting distinct resistance patterns and gene prevalence. It offers updated evidence on the dominance of blaZ and absence of vanA in bovine *S. aureus*, supporting targeted antimicrobial stewardship in dairy herds.

Material and Methods

Collection of milk samples

Milk samples were collected randomly from organized dairy herds. The udder and teats of cows were clean dried thoroughly and about 10 ml of quarter foremilk sample was collected from each

quarter into sterilized 15 ml glass tubes as per standard microbial procedures of National Mastitis Council.

Isolation of *S. aureus*

The milk samples were streaked on blood agar plates and incubated at 37^{0}C for 18-24 hours. The individual bacterial colonies were noted and presumptively staphylococci were identified based on colony morphology, gram staining characteristics and positive catalase test. The *Staphylococcus aureus* was differentiated from other *Staphylococcus* spp. based on colony morphology (round, raised, opaque, yellow to golden yellow colonies of 1-2mm in diameter with or without beta hemolysis) on blood agar and yellow pigmentation on mannitol salt agar (*S. aureus* is mannitol fermenting). The final confirmation of *S. aureus* was done through PCR (amplification of *16SrDNA* and *nuc* gene).

Antibiotic resistance testing by agar disc diffusion

Antibiotic sensitivity testing of *S. aureus* was performed on Mueller-Hinton agar (HiMedia) using the disc diffusion method (Quinn et al. 2004) following guidelines of Clinical and Laboratory Standards Institute (CLSI 2018). Fifteen commercially available antibiotics (HiMedia discs) belonging to 8 antimicrobial classes were evaluated. Briefly, 3-5 colonies of *S. aureus* were inoculated in 5ml of trypticase soy broth (TSB) and incubated at 37^{0}C for 2-6 hours, till inoculum turbidity reached 0.5 McFarland standard (HiMedia Laboratories Pvt. Ltd., Mumbai, India). This inoculum was streaked over the surface of Muller Hinton Agar (MHA) plates using a sterile disposable cotton swab squeezing surplus inoculum. *S. aureus* strain ATCC 25923 was used as a control strain. The discs were firmly placed on agar surface by means of sterile forceps and plates were incubated for 16-18 hours at 37^{0}C. The diameters of growth of inhibition were measured in millimetres and reported as susceptible, intermediate or resistant. The phenotypic antibiotic resistance profile of MRSA and MSSA isolates against different antimicrobial classes is presented in Table 1.

PCR detection of antibiotic resistance genes

DNA extraction

After overnight inoculation of an individual bacterial colony in brain heart infusion broth (BHI, HiMedia), 1 ml of broth was pelleted at 7500 rpm for 5 minutes in refrigerated centrifuge (Heraeus Biofuge Primo R, Thermo Scientific). The pellet was suspended in 180 µl lysis solution (lysozyme enzyme 20 mg ml^{-1}; Tris HCL 20 mM, pH 8, Triton X 1.2%; Tween 20 0.5% and EDTA 2 mM) and incubated at 37 ^{0}C for 30 min. Bacterial DNA was extracted using QIAamp DNA mini kit (Qiagen) as per manufacturer guidelines. Eluted genomic DNA was stored at -20 ^{0}C until use.

PCR detection of methicillin resistant gene (*mecA*) for MRSA

The PCR assay involved 12.5 ul of Taq DNA Polymerase Master Mix (G-biosciences), 10 pmol/ul of each primer set containing forward and reverse primers, 2ul DNA template and sterilized nuclease free water to make up a total of 25µl reaction volume. The amplification involved an initial denaturation at 94$^{Ë%}$C for 3 minutes; followed by 30 cycles each of denaturation at 94$^{Ë%}$C for 30 seconds; annealing at 51$^{Ë%}$C for 30 seconds and extension at 72$^{Ë%}$C for 1 minute; followed by a final extension at 72$^{Ë%}$C for 5 minutes and hold at 4$^{Ë%}$C.

PCR Amplification of coagulase gene (coa)

Isolates were tested for the presence of *coa* gene as per protocol of Goh et al. (1992). The PCR mixture was prepared in 25µl reaction and amplified using the following thermos cycling programme: initial denaturation at 95$^{Ë%}$C for 3 minutes, followed by 30 cycles each of denaturation at 95$^{Ë%}$C for 30 seconds, annealing at 55$^{Ë%}$C at 30 seconds, extension at 72$^{Ë%}$C for 1 min and final extension at 72$^{Ë%}$C for 5 minutes.

PCR for detection of µ§-lactamase gene (*blaZ*), tetracycline resistant gene (*tetK* and *tetM*) and aminoglycoside resistant gene (*aacA-aphD*).

Conventional single plex PCR was optimized for the amplification of four antibiotic resistant genes using primer pairs mentioned in Table 2. PCR amplification of these genes was carried out in a reaction of 25µl consisting of 12.5µl of Taq DNA Polymerase Master Mix (G-biosciences), 10 pmol/ul of each primer set containing forward and reverse primers, 2ul DNA template and sterilized nuclease free water to complete the reaction volume. The cycling conditions were: initial denaturation at 94$^{Ë%}$C for 5 minutes, followed by 30 cycles each of denaturation at 94$^{Ë%}$C for 30 seconds, annealing at 50$^{Ë%}$C at 45 seconds (*blaZ*); 49$^{Ë%}$C for 30 seconds (*tetK* and *tetM*); 51$^{Ë%}$C for 30 seconds (*aacA-aphD*), followed by extension at 72$^{Ë%}$C for 1 min and final extension at 72$^{Ë%}$C for 5 minutes.

PCR for detection of erythromycin resistance gene (*ermA*) and vancomycin resistance gene (*vanA*)

Conventional single plex PCR was optimized for the amplification of erythromycin resistance gene (*ermA*) and vancomycin resistance gene (vanA) with primer pairs given in table 2. The PCR mixture was prepared in 25µl reaction consisting of 12.5µl of Taq DNA Polymerase Master Mix (G-biosciences) and amplified using the following thermos cycling programme: initial denaturation at 94$^{Ë%}$C for 5 minutes, followed by 30 cycles each of denaturation at 94$^{Ë%}$C for 30 seconds, annealing at 55$^{Ë%}$C at 40 seconds (*ermA*) and 54$^{Ë%}$C for 45 seconds (*vanA*); followed by

Table 1: Phenotypic antibiotic resistance pattern in MRSA and MSSA

Antimicrobial classes	Antibiotic disk (content)	Interpretation	*S. aureus* (n=103)	MRSA (n=61)	MSSA (n=42)
β-Lactams (Penicillins and cephalosporins)	Penicillin-G (P-10)	S	7 (6.79 %)	0 (0.00 %)	7 (16.66 %)
		I	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)
		R	96 (93.20 %)	61 (100 %)	35 (83.33 %)
	Ampicillin (AMP-10)	S	33 (32.03 %)	7 (11.47 %)	26 (61.90 %)
		I	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)
		R	70 (67.96 %)	54 (88.52 %)	16 (38.09 %)
	Amoxicillin (AMX-10)	S	37 (35.92 %)	7 (11.47 %)	30 (71.42 %)
		I	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)
		R	66 (64.07 %)	54 (88.52 %)	12 (28.57 %)
	Ceftriaxone (CTR-30)	S	35 (33.98 %)	3 (4.92 %)	32 (76.19 %)
		I	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)
		R	68 (66.02 %)	58 (95.08 %)	10 (23.81 %)
	Cefoperazone (CPZ-75)	S	48 (46.60 %)	13 (12.62 %)	35 (83.33 %)
		I	3 (2.91 %)	2 (3.28 %)	1 (2.38 %)
		R	52 (50.48 %)	46 (75.41 %)	6 (14.28 %)
β-Lactam Combination	Amoxycillin + Clavulanate (AMC-30)	S	66 (64.07 %)	28 (45.90 %)	38 (90.47 %)
		I	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)
		R	37 (35.92 %)	33 (54.09 %)	4 (9.52 %)
	Ceftriaxone + Sulbactum (CIS-30)	S	51 (49.51 %)	13 (21.31 %)	38 (90.47 %)
		I	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)
		R	52 (50.48 %)	48 (78.68 %)	4 (9.52 %)
Cephamycins	Cefoxitin (CX-30)	S	42 (40.77 %)	0 (0.00 %)	42 (100 %)
		I	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)
Cephamycins	Cefoxitin (CX-30)	R	61 (59.22 %)	61 (100 %)	0 (0.00 %)
		S	81 (78.64 %)	40 (65.57 %)	41 (97.62 %)
		I	0	(0.00 0	(0.00 0 (0.00

			%)		%)		%)	
		R	22	(21.36	21		1	(2.38
			%)		(34.43 %)		%)	
	Levofloxacin	S	68	(66.02	31		37	
	(LE-5)		%)		(50.82 %)		(88.09 %)	
		I	15	(14.56	12		3	(71.42
			%)		(19.67 %)		%)	
		R	20	(19.42	18		2	(4.76
			%)		(29.51 %)		%)	
		S	41	(39.80	13		28	
Macrolide	Erythromycin		%)		(21.31 %)		(66.66 %)	
	(E-15)	I	53	(51.45	39		14	
			%)		(63.93 %)		(33.33 %)	
		R	9	(8.73	9	(14.75	0	(0.00
			%)		%)		%)	
	Tetracycline	S	80	(77.66	41		39	
Tetracyclines	(TE-30)		%)		(67.21 %)		(92.85 %)	
		I	9	(8.73	7	(11.47	2	(4.76
			%)		%)		%)	
		R	14	(13.59	13		1	(2.38
			%)		(21.31 %)		%)	
	Gentamicin	S	76	(73.78	39		37	
	(GEN-10)		%)		(37.86 %)		(88.09 %)	
Amino-glycosides		I	13	(12.62	10		3	(7.14
			%)		(16.39 %)		%)	
		R	14	(13.59	12		2	(4.76
			%)		(19.67 %)		%)	
	Kanamycin	S	31	(30.09	19		12	
	(K-30)		%)		(31.14 %)		(28.57 %)	
		I	11 (10.67 %)		5	(8.19	6	(14.28
					%)		%)	
		R	61	(59.22	37		24	
			%)		(60.65 %)		(57.14 %)	
Folate pathway	Cotrimaxazole	S	89	(86.40	49		40	
inhibitors	(COT-25)		%)		(80.32 %)		(95.23 %)	
		I	5	(4.85	3	(4.92	2	(4.76
			%)		%)		%)	
		R	9	(8.73	9	(14.75	0	(0.00
			%)		%)		%)	

extension at 72ËºC for 1 min and final extension at 72ËºC for 5 minutes.

Results and Discussion

Prevalence of S. aureus in milk samples

A total of 480 cow quarter milk samples were collected at random from 120 cows from different farms and were subjected to CMT and microbiological examination. Out of 480 quarters, 185 had CMT score > 1 out of which 115 had growth on microbial culture indicating specific subclinical mastitis and 70 had no growth suggestive of non-specific mastitis. 125 quarters had CMT score less than 1 but revealed growth on microbial culture indicating latent infection. 50 quarters had clinical mastitis evident from the physical changes in milk and out of them, only 68 % (34/50) quarters revealed growth on microbial culture. 120 were healthy quarters. Out of total 194 Staphylococci spp. isolated from milk, 173 were isolated from specific subclinical mastitis and latent infection and 21 Staph spp. were isolated from clinical mastitis. The prevalence of *S. aureus* in specific subclinical mastitis + latent infection was 52.6 % (91/173). The prevalence of *S. aureus* in specific clinical mastitis was 57.1 % (12/21). The overall prevalence of *S. aureus* was 53.1 % (103/194). Studies have reported *S. aureus* in 24.2 to 27.1 % of subclinical mastitis cases (Sommerhauser et al. 2003). In clinical mastitis, Duse et al. (2021) reported S. aureus as the most common mastitis pathogen accounting for 27.8 % of diagnosis.

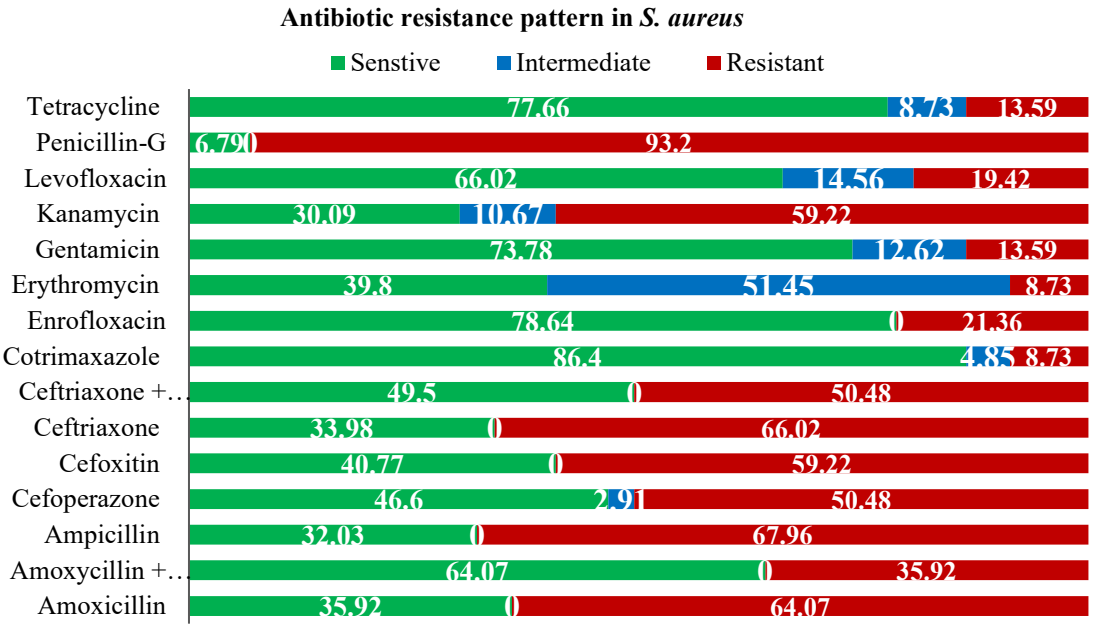

Fig. 1. Antibiotic resistance pattern in *S. aureus* (n=103)

Table 2: MAR indices of MRSA and MSSA isolates

MAR index	MRSA (n=61)	Percentage	MSSA (n=42)	Percentage
00	0	0.00 %	3	7.14 %
0.1	0	0.00 %	20	47.61 %
0.2	0	0.00 %	10	23.81 %
0.3	5	8.19 %	5	11.90 %
0.4	5	8.19 %	4	9.52 %
0.5	18	29.51 %	0	0.00 %
0.6	11	18.03 %	0	0.00 %
0.7	15	24.59 %	0	0.00 %
0.8	2	3.27 %	0	0.00 %
0.9	5	8.19 %	0	0.00 %
1.0	00	0.00 %	0	0.00 %

Phenotypic antibiotic resistance profile

S. aureus isolates were categorized into two: methicillin-resistant S. aureus (MRSA) and methicillin-susceptible *S. aureus* (MSSA). Isolates showing resistance to cefoxitin were classified as methicillin resistant *S. aureus* (MRSA phenotypically) and those showing susceptibility to cefoxitin were noted as methicillin-susceptible *S. aureus* (MSSA). Antibiotic resistance pattern was then evaluated accordingly. 61 (59.22 %) S. aureus isolates were phenotypically identified as MRSA and 42 (40.77 %) were MSSA. Out of 103 isolates, highest resistance was observed for penicillin (93.20%) with only 6.76% showing susceptibility to penicillin. Our findings are in agreement with other researchers (Hendriksen et al. 2008; Shi et al. 2010 and Ramdan et al. 2017) who performed the antibiogram of S. aureus and found highest resistance for penicillin. Methicillin-resistant S. aureus (MRSA), which can also be called multidrug-resistant Staphylococcus aureus, is defined as any strain of S. aureus that has developed resistance to all beta-lactam antibiotics, including the penicillins (methicillin, dicloxacillin, oxacillin, etc.) and the cephalosporins (Musser and Kapur 1992).

In our study, MRSA showed 100% resistance to penicillin., followed by 95.02 % resistance to ceftriaxone, followed by equal resistance to ampicillin and amoxycillin (88.52% each) and 75.41% resistance to cefoperazone. Among MSSA, highest resistant (83.33 %) was shown to penicillin. High resistance to penicillin in MRSA has also been found in other studies. Bhat et al. (2017) reported 100 % resistance to penicillin in MRSA and Mushtaq et al. (2019) reported 94.12 % resistance to penicillin in MRSA. Similar high resistance to ampicillin and amoxycillin was also recorded by Hamid et al. (2017) who reported 83.3% resistance to ampicillin and amoxicillin. For other µ§-Lactam antibiotics, MSSA showed 38.09% resistance to ampicillin, 28.57 % resistance to amoxicillin, 23.81 % resistance to ceftriaxone and 14.28 % resistance to cefoperazone. Comparatively, MRSA

showed higher resistance to all µ§-Lactam antibiotics. MRSA showed high resistance to µ§-Lactam combinations, Amoxycillin + clavulanic acid (AMC; 54.09%) and Ceftriaxone + Sulbactam (CIS; 78.68%). Alternately, MSSA showed high susceptibility (90.47 % each) towards both µ§-Lactam combinations (AMC and CIS). Kumar et al. (2010) had similar findings with resistant percent between MRSA and MSSA for beta lactam antibiotics being penicillin-G (100, 13.9), cloxacillin (100, 5.2), ampicillin (100, 6.0), amoxycillin (100, 4.3), amoxycillin-sulbactum (100, 2.6) and amoxicillin-clavulanate (76.9, 2.6). Among MRSA and MSSA isolates, the resistant percent proportion to aminoglycosides group (presented in parenthesis in percent) was gentamycin (38.4, 29.6), streptomycin (23.1, 27.0) and kanamycin (30.8, 25.2). Moreover, MRSA isolates also showed higher resistance percent to other antibiotics as compared to MSSA: cefixime The overall antibiotic resistance pattern of *S. aureus* isolates is illustrated in Fig. 1. (23.1, 19.1), ciprofloxacin (30.8, 12.1), ofloxacin (23.1, 5.2), clindamycin (30.8, 12.2), lincomycin (23.1, 15.7), pristinomycin (23.1, 7.0), tetracycline (53.8, 34.8), erythromycin (38.5, 20.9), rifampicin (23.1, 6.1), and chloramphenicol (38.5, 15.7).

Both MRSA and MSSA did not show high resistance to floroquinolones. Our findings corroborate with Chandrasekaran et al. (2014) and Mushtaq et al. (2019) who found that MRSA and MSSA were sensitive to fluoroquinolones. Among MRSA, resistance to levofloxacin was 21.31% and to enrofloxacin was 34.43 %, while among MSSA there was high susceptibility towards levofloxacin (88.09 %) and enrofloxacin (97.62 %). MRSA showed low resistance towards erythromycin (14,75 %), cotrimaxazole (14.75 %) and tetracycline (21.3 %). Similarly, among MSSA no isolate showed resistance to erythromycin and

Table 3: Prevalence of antibiotic resistant genes and coagulase positive gene in MRSA and MSSA isolates

S. No.	Resistance genes	MRSA (n=61)	Percentage	MSSA (n=42)	Percentage
1	*mecA*	38	62.29 %	0	0.00 %
2	*blaZ*	24	39.34 %	8	19.04 %
3	*aacA-aphD*	18	29.51 %	7	16.66 %
4	*tetK*	14	22.95 %	3	7.14 %
5	*tetM*	6	9.83 %	0	0.00 %
6	*ermA*	4	6.55 %	1	2.38 %
7	*vanA*	0	0.00 %	0	0.00 %
8	*Coa*	35	57.37 %	14	33.33 %

Table 4: Distribution of phenotypic and genotypic antibiotic resistance pattern in *S. aureus*

Antibiotic	Gene of interest	Antibiotic resistance		
		Phenotypic		Genotypic
Cefoxitin	*mecA*	S	42	0
		I	0	0
		R	61	38
Penicillin	*blaZ*	S	7	0
		I	0	0
		R	96	32
Gentamicin	*aacA-aphD*	S	76	11
		I	13	4
		R	14	10
Tetracycline	*tetK*	S	80	2
		I	9	5
		R	14	10
	tetM	S	80	0
		I	9	0
		R	14	6
Erythromycin	*ermA*	S	41	1
		I	53	1
		R	9	3
Levofloxacin	*norA*	S	68	24
		I	15	7
		R	20	8

cotrimaxazole. Also, only 1 isolate showed resistance to tetracycline and 92.85 % were susceptible to it. Within aminoglycosides, resistance to gentamicin was low both in MRSA (19.67 %) and MSSA (4.76 %). However, kanamycin resistance was high in MRSA (60.65 %) and MSSA (57.14 %). The distribution of multiple antibiotic resistance (MAR) indices among MRSA and MSSA isolates is presented in Table 2. Our findings are more or less in agreement with Shah et al. (2019) who recorded high sensitivity against vancomycin (88.57%), gentamicin (84.2%), ciprofloxacin (82.8%), amoxicillin (72.85%), tetracycline (68%), amikacin (61.42%) and enrofloxacin (60%). They reported high resistance against penicillin (99.2%), ampicillin/salbactam (85.7%), kanamycin (71.42%), cefotaxime (54.28) and sulphadizine (51.48%).

Prevalence of antibiotic resistance genes

Detection of genotypic resistance to methicillin was carried out by PCR amplification of *mecA* gene and isolates showing a 533 bp band were confirmed genotypically positive MRSA. The primary resistance mechanism for MRSA is determined by the expression of the gene *mecA* which encodes PBP2a, a penicillin-binding protein (PBP) with low affinity than normal PBPs, which results in resistance to all ß-lactam antimicrobials through horizontal gene transfer (Musser and Kapur 1992).

The prevalence of mecA gene in MRSA was 62.29 % (38/61). Hamid et al. (2017) recorded a lower prevalence (20 %) of mecA in phenotypically positive MRSA. MSSA isolates did not carry mecA. The prevalence of antibiotic resistance genes in MRSA and MSSA isolates is summarized in Table 3. Among MRSA, highest prevalence was of blaZ (39.34 %), followed by aacA-aphD (29.51 %), tetK (22.95 %), tetM (9.83 %) and ermA (6.55%). In our study, tetK prevalence was higher than tetM which is in agreement with Kumar et al. (2010) who found that the occurrence of *tet*K (30. 5%) among the isolates was significantly higher than *tet*M (3.9%) gene. Similar pattern was present in MSSA showing highest prevalence of blaZ (19.04 %) and lowest of ermA (2.38 %). Our findings corroborate with Jamali et al. (2014) who reported most common antibiotic resistance genes to be blaZ (39.5 %). Ardic et al. (2005) noted that *tetM* and ermA genes were found in a higher percentage of MRSA (50.0%). The overall prevalence of coagulase gene coa was 47.57 % (49/103) in S. aureus. MRSA showed higher prevalence of coa gene (57.37 %) than MSSA (33.33 %) (Brahma et al. 2022; Singh, A. 2019).

Relation between phenotypic and genotypic antibiotic resistance in *S. aureus*

Out of 103 isolates, 61 were resistant to cefoxitin and identified as MRSA phenotypically. The relationship between phenotypic resistance and corresponding antibiotic resistance genes is presented in Table 4. However, only 62.29 % (38/61) carried the mecA gene primarily responsible for methicillin resistance (Shilpa, B. 2021). Our findings corroborate with that of Mushtaq et al. (2019) who observed the presence of mecA gene only in 7 (41.17 %) out of 17 MRSA detected phenotypically. Out of 96 isolates which were phenotypically resistant to penicillin, genotypic resistance mediated by blaZ gene was present only in 33.33 % (32/96) of them. Out of 14 isolates which were resistant to gentamicin, 71.43 % (10/14) carried aacA-aphD gene. 76 isolates were susceptible to gentamicin and out of them 14.47 % (11/76) isolates carried aacA-aphD gene. Among tetracycline resistant genes, tetK had higher prevalence than tetM in tetracycline resistant S. aureus. Out of 14 tetracycline resistant isolates, tetK was present in 71.42 % (10/14) and tetM was present in 42.85 % (6/14) isolates. 80 isolates were phenotypically susceptible to tetracycline, out of which only 2.5 % (2/80) carried tetK gene. TetM was not present in tetracycline susceptible isolates. Only 8.73 % (9/103) isolates were phenotypically resistant to erythromycin out of which 33.33 % (3/9) carried ermA gene. 20 isolates were resistant to levofloxacin out of which 40 % (8/20) isolates carried norA gene. 68 isolates were phenotypically susceptible to levofloxacin and out of them 35.29 % (24/68) carried norA gene (Ali, 2020).

Conclusion

The present study concluded that in MRSA, highest resistance was present for penicillin and cephalosporin group of antibiotics and lowest resistance was present for co-trimoxazole, enrofloxacin, gentamicin and tetracycline. Among MRSA, antibiotic resistance gene of highest prevalence was blaZ. All MRSA isolates were resistant to atleast one group of antibiotic suggesting high risk of antibiotic exposure and a wide range of antibiotic resistance among MRSA as compared to MSSA.

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