Molecular epidemiology and associated risk factors of nasal carriage of *Staphylococcus aureus* among health care workers in Salem, Tamil Nadu, India

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Abstract
Commonly Staphylococcal aureus causes infections in children or hospitalized patients. They continue to suffer, in spite of giving antibiotic treatment and have long been recognized as a major health issue. In contemporary times, most of the superficial and systemic infections are attributable to methicillin-resistant and multi-drug resistant Staphylococcus aureus (MDRSA), posing significant challenges for clinicians in managing their treatment. Healthcare workers are one of the major reservoirs of S. aureus. Screening of nasal carriers among healthcare workers is an important component in controlling and in prevention. A cross-sectional study involving 217 healthcare workers including physicians, nurses, technicians and ward attendees, was conducted. The collected nasal swab samples inoculated on Mannitol salt agar, nutrient agar, blood agar. The virulence factors of the isolated bacterial strains were phenotypically and genotypically determined.

The virulent strains were genotypically identified by sequencing their 16s rRNA gene followed by phylogenetic analysis. Kirby-Bauer method was employed to check the antibiotic sensitivity of the isolates. Among the 576 bacterial isolates, 124 were found to be Staphylococcus sp. and 12 were identified as S. aureus both phenotypically and genotypically. Antibiotic sensitivity patterns revealed the prevalence of S, aureus isolates sensitivity (62.87%), resistance (37.12%), MRSA (66.66%) and multi drug resistance (MDR; 58.33%). This study revealed the prevalence of asymptomatic carriage found with virulent, MRSA and MDR S. aureus isolates.

Keywords: Nasal carriage, Multidrug resistance *Staphylococcus aureus*, Coagulase, MRSA, Virulence factors.

Introduction

The incidence of asymptomatic nasal carriage of *Staphylococcus aureus* is found among 30% of the humans[26]. Occurrence of nasal carriage is mainly due to the establishment of solid interactions of *S. aureus* with nasal epithelial cells followed by overcoming host defence. Certainly, host characteristics and environment are the key factors that can predispose to colonization. Some factors favour *S. aureus* colonization and sometimes prevent colonization[31] by innate defence mechanisms[27]. The exploration of the relationship between aggressive strains and the host microflora is delving deeper and becoming more complex, revealing the involvement of other beneficial microorganisms in influencing the colonization of *S. aureus*[17]. Colonization might be harmless; however, it could be a risk factor for developing subsequent infections[7].

The evading immune mechanism in *S. aureus* reflects the interaction of bacteria with host through insistent growth and colonization and recurrent invasive infections[37]. The development of nose colonization in the human is due to inconsistency in host adhesins, immune response by secreting antimicrobials.[24] The presence of bacteria in the nose is connected to hand transmission as hands serve as the primary mode of transferring the pathogen between the area where one picks their nose (anterior nares) and the surfaces[30].

S. aureus, a versatile bacterial pathogen, has emerged as a significant concern in healthcare settings because of their potent to induce a variety of infections, spanning from skin and soft tissue infections to critical life-threatening conditions like bloodstream infections, endocarditis and osteomyelitis.[27,36,38] The virulent *S. aureus* produces several enzymes[34] such as coagulase[6], lipase[35], hyaluronidase[19], staphylokinase, nuclease and toxins[34] including staphylococcal enterotoxins[18], exfoliative toxins[23], toxic shock syndrome toxin[30], P-V Leucocidin [14], α –toxin, β – toxin and δ-toxin and biofilms[20]. Through these virulence factors, *S. aureus* persist, invading to cause cells and tissue damage and manifests various infections.

Indiscriminate usage of antibiotics in treating various diseases often results in the development of antimicrobial resistance[32] amongst bacterial strains. Antibiotic resistance *S. aureus* is considered as a global threat[12] and the arrival of virulent MDRSA is a key factor for the increased morbidity rates and difficulties in their treatment[15], in particular methicillin-resistant *S. aureus* (MRSA) strains.[42] The increasing occurrence of MRSA strains, among HCWs globally, poses a burgeoning public health issue.[13] Moreover, lifestyle factors could increase *S. aureus* carriage and antibiotic resistant profile of the *S. aureus* isolates.[9] In asymptomatic carriers who harbor multi-drug resistant bacterial strains, there is a possibility of *in vivo* transfer of

antibiotic-resistant genes to normal microbial flora posing a hazard to human-kind[32], as treating them is difficult for many other infections and may spread MDR strains easily to others.

HCWs are at the forefront of patient care and asymptomatic carriage of *S. aureus* strains among them has been identified as a significant risk factor for its transmission[10]. Auto-infections among carriers or the transmission bacteria from HCWs by contact or through fomites[27] serve as vectors[3] to patients[41], leading to increase morbidity in the vulnerable patients. Understanding the epidemiology of virulent *S. aureus* nasal carriage among HCWs is crucial for both infection control measures and patient safety[3,25,28]. Thus, the current study is focused on the nasal carriage of *S. aureus*, associated virulence factors and their antibiotic sensitivity patterns amongst HCWs, thereby control measures can be suggested to prevent staphylococcal infections.

Material and Methods

Study design and population: Totally, 217 healthcare workers were involved in the cross-sectional study in Salem dt, Tamil Nadu. The participants' socio-demographic characteristics and associated risk factors were encompassed in this study. Consequently, the patient's data were collected during nasal swab sampling through structured questionnaires on demographic variables, antibiotic use and COVID-19 vaccination. This study was conducted from 01.09.2023 to 30.05.2024.

Inclusion and exclusion criteria: All healthcare workers coming under the inclusion criteria were asymptomatic and COVID-19 vaccinated. Exclusion criteria included those who were found to have a certain sort of respiratory illness and no COVID-19 vaccinated. All the participants involved in this study provided informed written consent.

Sample collection and processing: A total of 217 nasal swabs were obtained from healthcare workers using sterile swab which was then placed into nutrient broth (Himedia, India) and transported to the lab in an ice box. The broth was serially diluted in peptone broth and incubated for 5 h. Then the peptone broth was inoculated on nutrient agar plates and incubated at 37 ºC for 24 h. The bacterial isolates, distinguished by unique colony morphology, underwent four successive subcultures to achieve pure cultures for each isolate. These pure cultures were then preserved in glycerol stock for future applications.

Phenotypic identification of bacterial isolates: The isolates were subjected to preliminary tests including Gram's staging, catalase and oxidase test for initial screening of *Staphylococcus* sp. Further, the isolates were tested for biochemical characteristics using a sugar fermentation medium viz. glucose, lactose, mannitol, sucrose, IMViC (Indole, methyl red, Voges Proskauer, Citrate utilization), TSI (Triple sugar iron) and urease. Colony characteristics were also studied using blood agar and mannitol salt agar[33].

Genotypic identification of bacterial isolates: All the 12 phenotypically identified bacterial isolates were subjected for genomic DNA isolation by phenol-chloroform method and fragmented by gel electrophoresis using 1 % agarose. The 16S rRNA gene of the bacterial isolates was amplified by universal primers, 27F and 1492R at the standard PCR conditions. The amplified products were purified using the QIA-quick PCR extraction kit (QIAGEN) and submitted for sequencing.

Further, the sequences underwent analysis through BLASTn and aligned using reference sequences with the help of ClustalW. Phylogenetic trees constructed using the aligned 16S rRNA gene sequences by neighbor-joining method in Mega X, with bootstrap analysis. The 16S rRNA gene sequences of 12 *S. aureus* isolates were submitted to NCBI GenBank to obtain a unique accession number for each isolate.

Phenotypic virulence characteristics: Coagulase activity[33], hemolysis[11], lipase production[33], protease production[33], DNase activity[33] and biofilm production[5; 11] were performed for the *S. aureus* isolates.

Genotypic virulence characteristics: All 12 *S. aureus* isolates' genomic DNA were used with primers (Table 1) for the detection of the genes encoding for the virulence characteristics including coagulase (*Cog*), hemolysins (*hlg*), fibrinolysin (*fnbA)*, climbing factor(*clfA*), toxic shock syndrome toxin (*tst*) and enterotoxin (*se*b). The specific primers used in this study were listed in table 1 and PCR amplification of the specific virulence genes afore-mentioned with PCR conditions. The amplified PCR products were analyzed for their quality and molecular weight of each specific virulence gene was determined using 1.2% agarose gel electrophoresis with a specific standard DNA ladder.

Antibiotic sensitivity pattern of *S. aureus* isolates: The isolated Staphylococcal strains were evaluated for antibiotic sensitivity pattern using the following antibiotics (Hi Media, India): co-trimoxazole (20 µg), rifampicin (5 µg), vancomycin (10 µg), chloramphenicol (25 µg), streptomycin (10 µg), ampicillin (10 µg), cefotaxime (10 µg), methicillin (10 µg), amikacin (30 µg), erythromycin (15 µg), cefixime (30 µg), gentamycin (20 µg) by disc diffusion assay. Briefly, pure colonies of each isolated bacterial strain from nutrient agar were inoculated into tryptone-soya broth (Hi Media, India) and incubated at 37 °C until broth reached 0.5 McFarland standard. Using a sterile swab, the culture broth suspension was inoculated on the surface of the Mueller Hinton agar plate. The discs of each antibiotic were positioned on the MHA plate and incubated over night at 37 °C and the size zone of inhibition was measured and reported according to CLSI[8].

Table 1
Primers used in the virulence characteristics study of *S. aureus* isolates

Gene(s)	primer	Nucleotide sequences	Size of the amplicon	No. of nucleotides
Coagulase encoding gene (coag)	coag-F	5' -ACCACAAGGTACTGAATCAACG-3'	812	22
	coag-R	5' -TGCTTTCGATTGTTCGATGC-3'		20
Hemolysin encoding gene (hlg)	hlg-F	5'-GCCAATCCGTTATTAGAAAATGC-3'	938	23
	hlg-R	5' -CCATAGACGTAGCAACGGAT-3'		20
Fibrinogen binding protein A (fnbA)	fnbA-F	5' -CACAACCAGCAAATATAG-3'	1362	18
	fnbA-R	5' -CTGTGTGGTAATCAATGTC-3'		19
Clumping factors, A & B	clfA-F	5' -ATTGGCGTGGCTTCAGTGCT-3'	288	20
	clf-R	5' -CGTTTCTTCCGTAGTTGCATTTG-3'		23
Toxic shock syndrome toxin 1 (tsst-1)	tst-F	5' -CATCTACAAACGATAATATAAAGG-3'	476	24
	tst-R	5' -CATTGTTATTTTCCAATAACCACCCG-3'		26
Staphylococcal enterotoxin A	sea-F	5'-TGCAGGGAACAGCTTTAGGC-3'	250	20
	Sea-R	5' -GTGTACCACCCGCACATTGA-3'		20
Staphylococcal enterotoxin B	seb-F	5'-ATTCTATTAAGGACACTAAGTTAGGG-3'	400	26
	seb-R	5'-ATCCCGTTTCATAAGGCGAGT-3'		21

Statistical analysis: Chi-square test was employed to correlate association of socio-demographic characters and nasal carriage of CoPS and CoNs isolates using GraphPad Prism (Version 6, USA). *P*-value lesser than 0.05 was considered to indicate statistical significance.

Quality control: The data collection procedures, bacterial isolation, cultural and virulence characteristics, antibiotic sensitivity test and antibiotic discs were assessed for their performance and quality using the standard bacterial strain *Staphylococcus aureus* (ATCC 25923).

Ethical approval: The Ethical Committee approved this project (VMKVMC&H/IEC/24/058).

Results and Discussion

Sociodemographic characteristics of nasal carriers: The sociodemographic characteristics of the participants (n=217; Fig.1a,b) who were employed in the healthcare system such as hospitals, operation theatres and clinical laboratories including female (n=111), aged 36-50 (41%), married (75%), family size 3 (39%), degree holder (42%), technicians (33.64%), experience 5-10 years (41%), awareness of asymptomatic carriage (79%), known antibiotic usage (57.60%), were found to be dominant in this present study (Table 2). Similarly, asymptomatic carriers among the health workers have been conducted and reported in the countries including Tanzania[16], Ethiopia[43] and Iran[40].

Prevalence of nasal carriers of *S. aureus*: In this study, totally 576 bacterial isolates were isolated from 217 nasal swabs collected from different health care workers. Of these 124 bacterial isolates were screened as *Staphylococcus* based on the preliminary tests results (Gram-positive clusters, catalase positive and oxidase negative). The coagulase test was employed to differentiate coagulase-positive (CoPS) and coagulase-negative staphylococcus (CoNS). Among the 124 isolates, 12 staphylococcal strains were CoPS and remaining isolates (n=112) were CoNS. The 12 CoPS strains were biochemically identified as *Staphylococcus aureus* (Fig.1c). Further genotypic characterization through 16srRNA sequencing and phylogenetic analysis confirms the result of biochemical tests. The16s rRNA sequences of 12 *S. aureus* isolates were submitted to NCBI and their accession numbers and E value were given in table 3.

Based on the *S. aureus* isolates screened (SA-07, SA-24, SA-37, SA-68, SA-72, SA-91, SA-92, SA-96, SA-114, SA-138, SA-151, SA-174), the prevalence of asymptomatic carriage for *S. aureus* isolates was found to be 5.52 % (n=12) amongst healthcare workers. Among nasal carriers (CoPS) amongst male and female were 5 and 7 respectively. The rest of the isolates were found to be coagulase-negative *staphylococcus* (CoNS) (n=122) and non-staphylococci (n=444). Thus, prevalence rate of *S. aureus* nasal carriage was slightly higher in female cases (6.3 %) than male (4.7 %) among the HCWs. Conversely, Rongpharpi et al[30] reported that the prevalence of Staphylococcal nasal carriage is higher among female population than male candidates.

Peacock et al[24] stated that the development of *S. aureus* nasal colonization in the human carriage is due to variability in host adhesins, immunity and antimicrobial secretion. Observations included colonization resistance by *S. aureus*, along with the discovery that persistent carriers typically host a singular strain, whereas intermittent carriers may harbor unrelated strains over time. The present study also revealed the prevalence of CoPS, CoNS and non-Staphylococcus species amongst health care workers. Consequently, these individuals could serve as intermittent carriers, potentially transmitting the staphylococcal pathogens to the hospitalized patients and healthy visitors.

Virulence characteristics: The phenotypic characterization of CoPS *S. aureus* isolates (n=12) reveals a virulent

phenotype associated with a diverse array of virulence factors including hemolysins, lipases, proteases, DNases and biofilm-forming capacity.

Genotypic characterization revealed the presence of genes, *cog*, *hlg*, *fnbA*, *clfA*, *tst* and *seb* encoding coagulase, hemolysins, fibrinolysin, climbing factor, toxic shock syndrome toxin and enterotoxin respectively in all the 12 CoPS *S. aureus* strains. This indicates the virulence nature of isolated staphylococcal strains capable of causing mild to severe diseases which may lead to death.

The present study was not consistent with Vaez and Ghalehnoo[40] who reported the most common virulence factor is enterotoxin A followed by toxic shock toxin and enterotoxin C.

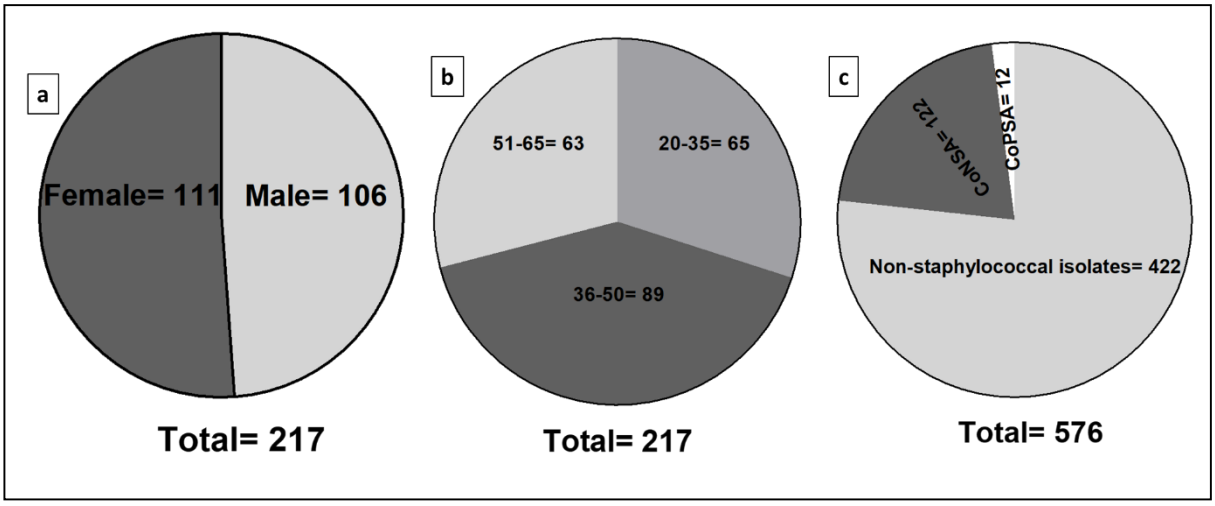

Figure 1: Details of nasal swab collected based on (a) sex, (b) age and (c) bacteria isolated from nasal swab

Table 2
Sociodemographic characteristics of nasal carriers amongst health care workers (*n*=217)

Characteristics	Category	Total No.	Frequency (%)
Sex	Male	106	49
	Female	111	51.15
Age	20-35	65	30
	36-50	89	41.01
	51-65	63	29.03
Marital status	Married	162	75
	Unmarried	55	25
Family size	2	52	24
	3	84	39
	3<	81	37
Education	10	24	11
	12	18	8
	Diploma	50	23
	Degree	92	42
	Masters	33	15
Occupation	Physician	64	29.49
	Nurse	56	25.80
	Technicians	73	33.64
	Ward attendees	24	11.05
Experience (Years)	1-3	70	32.25
	3-5	58	26.72
	5-10	89	41.01
Awareness of asymptomatic carriage	Yes	173	79.72
	No	44	20.27
Antibiotic use	Yes	125	57.60
	No	92	41.47

Table 3
A summary of the BLASTn analysis conducted on 12 *Staphylococcus aureus* isolates based on their 16S rRNA gene sequences.

Staphylococcal isolates	NCBI Accession no.	The isolates closely corresponded to species found in GenBank.	Percentage of query coverage	E value	Percentage of identity
CoPS07	OR958832	*Staphylococcus aureus* strain SU NSB4	99	0	99.31
CoPS24	OR960419	*Staphylococcus aureus* strain SSS B	99	0	98.34
CoPS37	OR960421	*Staphylococcus aureus* strain NCT45.4	100	0	98.93
CoPS72	OR960425	*Staphylococcus aureus* strain NSB 4	99	0	98.21
CoPS68	OR960426	*Staphylococcus aureus* strain DP05	99	0	98.60
CoPS91	OR960428	*Staphylococcus aureus* strain CIFRI:ST5	99	0	98.43
CoPS92	OR960486	*Staphylococcus aureus* strain SU NSB 4	99	0	98.4
CoPS96	OR960488	*Staphylococcus aureus* strain SAT2-19	99	0	97.99
CoPS114	OR960485	*Staphylococcus aureus* strain CoPSA24	99	0	97.82
CoPS138	OR960489	*Staphylococcus aureus* strain Sdc ortho23	99	0	98.25
CoPS151	OR960490	*Staphylococcus aureus* strain NCT45.4	99	0	97.80
CoPS174	OR960491	*Staphylococcus aureus* strain NCT45.2	99	0	97.9

These characteristics collectively contribute to the pathogenicity and clinical significance of staphylococcal infections, highlighting the importance of understanding and characterizing the virulence factors expressed by clinical isolates for effective management and treatment strategies.

In 2017, the World Health Organization (WHO) categorized MRSA as a high-priority pathogen, revealing its substantial impact on global health. This designation was made in recognition of the urgent need to develop effective treatments against MRSA infections, particularly those associated with biofilm formation[29]. Biofilms are assemblage of microbial communities enclosed with a self-secreted polymatrix, making them extremely resistant to antibiotics by inducing immune responses. Addressing the threat posed by MRSA biofilms is crucial for advancing sustainable therapeutic strategies and combating the growing challenge of antibiotic resistance[1].

Association of Socio-Demographic characteristics and nasal carriage of CoPS and CoNs: The current study found no statistically significant differences among sex (p = 0.537229), marital status (p = 0.075488), family size (p = 0.055685), experience (p = 0.112979) and antibiotic use (p = 0.784022) concerning the nasal carriage of Coagulase-positive *S. aureus* and Coagulase-negative *Staphylococcus*. However, it revealed highly significant associations with age (p = 0.003594), education (p = 0.000938), occupation (p < 0.00001) and awareness of asymptomatic carriage (p < 0.00001) for the nasal carriage of these bacteria (Table 4). In this line, Ohadian et al[22] observed a notable variance in nasal carriage of *S. aureus* across demographic variables. Specifically, differences were observed between sexes (P=0.035), hospitals (P=0.0001) and occupations (P=0.009). In addition, a significant discrepancy was identified between sexes (P=0.041) and occupations (P=0.034) concerning *S. aureus* carriage.

There were no significant differences found among the case of methicillin-resistant and multidrug-resistant patterns among the variables including sex (*P* = 0.624864), age (*P* = 0.657133), marital status (*P* = 0.1219), family size (*P* = 0.883642), education (*P* = 0.999661), occupation (*P* = 0.919493), experience (*P* = 0.974232), awareness of asymptomatic carriage (*P* = 0.4924) and antibiotic use (*P* = 0.917134) (Table 5).

Antibiotic sensitivity and resistant pattern: All 12 isolates of *S. aureus* underwent antibiotic sensitivity testing, with the results detailed in table 6. The study revealed varying degrees of sensitivity among the bacterial isolates to different classes of antibiotics, with rifamycin (91.66%)

exhibiting the highest sensitivity followed by chloramphenicol (75%), cephalosporin (66.66%), sulphonamides (66.66%), macrolide (66.66%), aminoglycoside (66.66%), β-lactam antibiotic (33.33%) and glycopeptide (58.3%).

Conversely, the isolates displayed the highest resistance to β-lactam antibiotic (66.66%) followed by glycopeptide (41.66%), aminoglycoside (33.33%), cephalosporin (33.33%), sulphonamides (33.33%), macrolides (33.33%), chloramphenicol (25%) and rifamycin (8.33%).

In addition to the mentioned results, the study identified 4 strains (33.33%) of *S. aureus* as methicillin-sensitive while 8 strains (66.66%) were methicillin-resistant, namely SA24, SA37, SA68, SA91, SA96, SA114, SA138 and SA51 (Table 6). Notably, 7 strains (58.33%) of *S. aureus*, specifically SA24, SA37, SA68, SA72, SA91, SA96 and SA114, were grouped as multidrug-resistant (MDR) based on the antibiotic sensitivity pattern. Ohadian et al[22] found that all MRSA isolates they examined, were susceptible to linezolid, fusidic acid and vancomycin. However, our current study reveals a diverse sensitivity pattern among MRSA strains towards other antibiotics used in this investigation.

Adhikari et al[2] reported a prevalence of MRSA at 26.4%. The MRSA isolates displayed complete resistance to cloxacillin and penicillin, followed by high resistance rates to ciprofloxacin, erythromycin, cephalexin, cotrimoxazole and clindamycin. Moderate resistance was observed against chloramphenicol and gentamicin. Furthermore, our study identified 4 strains (33.33%) of *S. aureus* as methicillin-sensitive while 8 strains (66.66%) were methicillin-resistant, specifically SA24, SA37, SA68, SA91, SA96, SA114, SA138 and SA51 (Table 6).

Table 4
Association of Socio-Demographic Characteristics and nasal carriage of *S. aureus* and virulence factor

Characteristics	Category	Prevalence no.	*Staphylococcus* isolates (no's)		Chi-square Value	*p-value*
			CoPS (n=12)	CoNS (n=122)		
Sex	Male	106	5	66	1.2427	.537229
	Female	111	7	56		
Age	20-35	65	2	16	15.6075	.003594*
	36-50	89	8	57		
	51-65	63	2	49		
Marital status	Married	162	7	78	5.1676)	.075488
	Unmarried	55	5	44		
Family size	2	52	4	21	9.2263	.055685
	3	84	3	36		
	3<	81	5	65		
Education	10	24	4	19	26.2861	.000938*
	12	18	2	28		
	Diploma	50	2	16		
	Degree	92	2	38		
	Masters	33	1	21		
Occupation	Physician	64	2	6	44.4407	< 0.00001*
	Nurse	56	4	48		
	Technicians	73	3	32		
	Ward attendees	24	3	36		
Experience (Years)	1-3	70	4	51	7.4714	.112979
	3-5	58	6	32		
	5-10	89	2	39		
Awareness of asymptomatic carriage	Yes	173	5	25	113.6849	< 0.00001*
	No	44	7	97		
Antibiotic use	Yes	125	8	73	0.4866	.784022
	No	92	4	49		

*Satistically significance

Table 5
Association of Socio-Demographic Characteristics and antimicrobial resistance *S. aureus*

	Category	Nasal Carriage (n=12)	Antimicrobial resistance (no's)		Chi-square Value	*p*-value
			MRSA	MDRSA		
Sex	Male	5	5	4	0.9404)	.624864
	Female	7	3	3		
Age``	20-35	2	2	2	2.4306	.657133
	36-50	8	3	3		
	51-65	2	3	2		
Marital status	Married	7	5	4	0.1219	0.1219
	Unmarried	5	3	2		
Family size	2	4	1	2	1.1661	.883642
	3	3	3	2		
	3<	5	4	3		
Education	10	4	3	2	0.6402	.999661
	12	2	2	2		
	Diploma	2	1	1		
	Degree	2	1	1		
	Masters	1	1	1		
Occupation	Physician	2	1	1	2.0022)	.919493
	Nurse	4	1	1		
	Technicians	3	3	2		
	Ward attendees	3	3	3		
Experience (Years)	1-3	4	2	2	0.4924	.974232
	3-5	6	4	3		
	5-10	2	2	2		
Awareness of asymptomatic carriage	Yes	5	2	3	0.709	.701512
	No	7	6	4		
Antibiotic use	Yes	8	5	4	0.173	.917134
	No	4	3	3		

Table 6
Antibiotic sensitivity and resistant profile of *S. aureus* isolates

S.N.	Class	Antibiotics	No. of isolates (%)	
			Sensitive	Resistant
1.	Aminoglycoside	Amikacin	8(66.66%)	4(33.33%)
2.		Gentamicin	9 (75%)	3(25%)
3.		Streptomycin	7(58.33%)	5(41.66%)
4.	β-lactam antibiotic	Methicillin	4(33.33%)	8(66.66%)
5.		Ampicillin	4(33.33%)	8(66.66%)
6.	Cephalosporin	Cefotaxime	8(66.66%)	4(33.33%)
7.	Chloramphenicol	Chloramphenicol	9(75%)	3(25%)
8.	Sulfonamides	Co-trimoxazole	8(66.66%)	4(33.33%)
9.	Macrolide	Erythromycin	8(66.66%)	4(33.33%)
10	Glycopeptide	Vancomycin	7(58.33%)	5(41.66%)
11.	Rifamycin	Rifampicin	11(91.66%)	1(8.33%)
Over all			83 (62.87%)	49 (37.12%)

Table 7
Multi drug resistant profile of *S, aureus* isolates

S.N.	Antibiotics	CoPS isolates											
		SA 07	SA 24	SA 37	SA 68	SA 72	SA 91	SA 92	SA 96	SA 114	SA 138	SA 151	SA 174
1.	Amikacin	S	R	S	R	S	R	S	R	S	S	S	S
2.	Gentamicin	S	S	R	R	S	R	S	S	S	S	S	S
3.	Streptomycin	R	R	S	R	R	R	S	S	S	S	S	S
4.	Methicillin	S	R	R	R	S	R	S	R	R	R	R	S
	Ampicillin	S	R	R	R	R	R	S	R	R	R	S	S
5.	Cefotaxime	S	R	S	R	S	S	S	R	R	S	S	S
6.	Chloramphenicol	S	R	S	S	S	S	R	S	S	S	S	R
7.	Co-trimoxazole	S	S	R	S	R	S	S	R	R	S	S	S
8.	Erythromycin	S	R	S	R	S	R	S	R	S	S	S	S
9.	Vancomycin	S	R	S	R	S	R	S	R	R	S	S	S
10.	Rifampicin	S	S	S	R	S	S	S	S	S	S	S	S
MDR status		-	MDR	MDR	MDR	MDR	MDR	-	MDR	MDR	-	-	-

Remarkably, 7 strains (58.33%) of *S. aureus*, particularly SA24, SA37, SA68, SA72, SA91, SA96 and SA114, were classified as multidrug-resistant (MDR) based on our analysis. Adhikari et al[2] noted a significantly elevated prevalence of multidrug resistance (MDR) in MRSA, reaching 94.05%, in contrast to 52.12% observed in methicillin-sensitive *S. aureus*. Similarly, our current investigation revealed that 75% of MRSA (n=6) were identified as multidrug-resistant (Table 7). The rapid emergence of antibiotic resistance among pathogens has created significant challenges within the medical community[4]. Staphylococci, for instance, can spread between individuals through both direct and indirect contact. The increasing occurrence of methicillin-resistant *S. aureus* (MRSA) and multi-drug resistant (MDR) strains poses a serious and growing concern in modern health[2].

Conclusion

The detection of these virulence genes suggests that the isolates possess a high level of pathogenicity, capable of causing a range of infections, varying from mild to severe. This susceptibility is influenced by host's immune response and the site of infection. These results highlight the critical need for continuous surveillance and implementation of effective infection control strategies to curb the dissemination of these highly virulent and MDR pathogens.

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