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Detection of Methicillin Resistance and Virulence Determinants in *Staphylococcus aureus* Isolated from Semen Samples

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Abstract

Staphylococcus aureus, a common bacterium of seminal fluid, is also potentially pathogenic, with virulence factors contributed by virulence genes. Bacteria have been shown to agglutinate and immobilize spermatozoa, affecting male fertility. Methicillin resistance in *S. aureus* has been a major concern in the treatment of diseases caused by *S. aureus*. This study aimed to detect methicillin resistance and its co-occurrence in the presence of virulence genes for clumping factors (*clf*A gene and *clf*B gene) and for toxic shock syndrome toxin (*tst* gene) in *S. aureus* isolates obtained from the semen of suspected infertile men of Nepal. This study included 21 isolates of *S. aureus* obtained from 217 semen samples of male partners among infertile couples visiting a fertility centre for consultation. A cefoxitin disc diffusion test following CLSI guidelines (2021) was conducted to screen for phenotypic methicillin resistance in the bacteria. DNA was extracted from the isolates to detect the *mec*A, *clf*A, *clf*B, and *tst* genes via PCR amplification using specific primer sets. The data obtained were analysed via Fisher's exact test at 95% confidence interval, with a *p-value* of <0.05 considered to be significant. Cefoxitin resistance was shown by 12 (57.14%) isolates, while the *mec*A gene was detected in 14 (66.67%) isolates. The odds of cefoxitin resistance were 6.25-fold higher among *mec*A-positive isolates than among *mec*A-negative isolates, although the association was not statistically significant (OR = 6.25, 95% CI: 0.68–57.2). The virulence genes *clf*A, *clf*B, and *tst* were present in 12 (57.14%), 12 (57.14%), and 2 (9.5%) of the isolates, respectively. There was no statistically significant correlation between the *mec*A gene and the virulence genes analysed in this study ($p > 0.05$). However, the detection of methicillin resistance in 66.67% of the isolates and at least one of the virulence genes of interest in 76.19% isolates reflects the antimicrobial resistance and virulence status of *S. aureus* in semen. Our study could not find significant association of methicillin resistance and virulence genes under study with semen quality (all $p > 0.05$). The study findings exhibit the presence of methicillin-resistant and virulence-associated *S. aureus* isolates in semen samples from suspected infertile men, which signifies the importance of microbiological investigation of semen during infertility evaluation. However, further studies are recommended to demonstrate the clinical significance of these isolates.

Keywords: infertility, methicillin resistance, semen, *Staphylococcus aureus*, virulence

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Introduction

Semen harbours different types of bacteria, from the genitourinary tract of men or sexually transmitted, including commensals, opportunistic pathogens, or potential pathogens [1,2]. Approximately 15% of male infertility is caused by infection of the genitourinary tract and male accessory glands. The presence of pathogenic bacteria in semen may cause an alteration in semen quality, decreasing the fertility potential of men. Bacteria in semen may lower the count, reduce viability and motility, affect morphology, or cause DNA damage to spermatozoa [3]. These effects of bacteria on semen characteristics have been demonstrated under *in vitro* and *in vivo* conditions in several studies [4].

Previously, semen was thought to be sterile in healthy male populations. However, studies on healthy and infertile men have shown the presence of various types of bacteria in both populations [5,6]. Skin commensals may be present in semen as contamination from the skin around the urethral opening [2]. According to the World Health Organization (WHO), bacteriospermia, or the presence of bacteria in semen, is significant if there is more than 10^3 bacteria/mL of ejaculate [7]. The bacteria commonly isolated from the semen of infertile men are *Enterococcus faecalis, Escherichia coli, Streptococcus agalactiae, Staphylococcus aureus, Corynebacterium, Pseudomonas,* and sexually transmitted bacteria influence the quality of semen and spermatozoa [8,9].

S. aureus, which is considered a commensal bacterium in humans, may also invade the reproductive tracts of men and women directly or via the hematogenous route [10]. *S. aureus* is also a common bacterium present in the semen of both fertile and infertile men [5]. The presence of *S. aureus* as the most abundant bacteria in the semen of men has been reported in several studies [1,11–13]. Although the presence of *S. aureus* in semen is also considered contamination from the skin of hands and

genitals, *in vitro* studies have also demonstrated deterioration in the quality of semen after the coincubation of semen and *S. aureus*. An *in vitro* study on bovine semen also revealed a decrease in sperm motility and increased production of reactive oxygen species (ROS), resulting in sperm DNA fragmentation after the coincubation of semen with different species of *Staphylococcus,* including *S. aureus* [14]. A reduction in the motility and agglutination of spermatozoa was observed after the coincubation of highly motile spermatozoa from normozoospermic men and live *S. aureus* isolated from the cervix of women with unexplained infertility [15]. *S. aureus* produces the extracellular metabolite sperm immobilizing factor (SIF), which can cause 100% spermatozoa immobilization and has a spermatogenic effect [16]. The incubation of sperm with a strain of *S. aureus* resulted in ultrastructural variation. Transmission electron microscopy revealed swollen mid-pieces and acrosomes of the spermatozoa after coincubation with *S. aureus* [17].

S. aureus in semen cannot be considered only a contamination, as studies have shown a deleterious effect on spermatozoal cells due to its presence in semen. Furthermore, *S. aureus* may harbour genes that confer its virulence and may also harbour antimicrobial resistance genes that help it combat antibiotics. This study was intended to detect virulence genes (*clf*A, *clf*B, and *tst*) and a methicillin resistance gene (*mec*A) in *S. aureus* isolated from semen of Nepalese men. The *clf*A and *clf*B genes encode for clumping factors, and play a role in fibrinogen-mediated adhesion and colonization, which may facilitate bacterial interaction with spermatozoa and reproductive tract epithelium [18,19]. The *tst* gene encodes for toxic shock syndrome toxin (TSST-1), a potent super antigen capable of inducing cytokine release and oxidative stress [20].

An infection caused by antibiotic-resistant and virulent organisms may be more difficult to treat because of limitation in the treatment option, and may also be due to enhanced abilities of bacteria for adhesion, colonization, and persistence within the male reproductive tract. Detection of MRSA in the male reproductive tract is therefore clinically important, as resistant strains may contribute to persistent genital colonization or infection and complicate infertility management.

An identification of resistant and virulent *S. aureus* strains could thus be important in microbiological investigation of suspected infertile men. We hypothesized that *S. aureus* isolates recovered from semen samples would frequently harbor methicillin resistance (*mecA*) and virulence genes (*clfA, clfB,* and *tst*), and that these determinants may occur together. To our knowledge, this is one of the first studies from Nepal to investigate methicillin resistance (*mecA*) and selected virulence genes (*clfA, clfB,* and *tst*) among *S. aureus* isolates recovered from semen samples. These findings provide baseline molecular epidemiological data that may support future investigations into the role of bacterial virulence and antimicrobial resistance in male infertility.

Materials and methods

Design, site, and population for the study

The study was cross-sectional on males among infertile couples visiting for fertility consultations at Creator's IVF Nepal Pvt. Ltd., Lalitpur, Nepal, from June 2021 to July 2022. The study was conducted after approval from the ethical review board of the Nepal Health Research Council (NHRC), Kathmandu, Nepal (Reg. no. 874/2019). The study population included male patients regardless of the cause and type of infertility after providing consent, along with their signatures. Patients receiving antibiotic therapy within the past five weeks were excluded.

Sample and laboratory analysis

Individuals of study population were requested to wash their hands and genitals, dry with a paper towel, and collect the samples (after sexual abstinence for 2–5 days) in a sterile leak-proof container (Tarsons, India) following the WHO criteria [21]. Semen quality was evaluated based on sperm concentration, total motility, morphology, and vitality according to WHO manual, 2021, and samples were categorized as normal or abnormal according to the WHO reference limits [22]. Samples were classified as having abnormal semen quality if one or more semen parameters were below the lower reference limits.

For microbiological investigation, samples (1 mL each in sterile microfuge tubes) were transported to the laboratory of the Central Department of Microbiology, Tribhuvan University, in an insulated ice box containing ice packs, maintaining a temperature of approximately 2–8°C until processing. A bacteriological study of the samples was performed within 3 h of sample collection, following the WHO criteria [21]. Semen culture was performed on MacConkey agar (MA), blood agar (BA), and chocolate agar (CA) (Hi-Media, India), and aerobic incubation was done at 37°C for 24 h. A significant growth of bacteria was considered only if the concentration was greater than 10^3 CFU/mL [7,8]. The isolated bacteria were identified based on growth

characteristics, microscopic morphology, and biochemical characteristics [23].

Identification of *S. aureus* and phenotypic methicillin resistance detection

S. aureus was identified as gram-positive cocci in clusters, catalase producers, oxidase non-producers, fermentative bacteria in the oxidative-fermentative test, coagulase producers, and DNase enzyme producers. The identification of *S. aureus* was done by phenotypic characteristics only. However, molecular confirmation could further improve species identification accuracy. The methicillin resistance of the isolates was detected by performing an antimicrobial susceptibility test of the isolates against the cefoxitin 30 mcg disc via the modified Kirby–Bauer disc diffusion technique [23]. The zone of inhibition (ZOI) (in mm), after incubation at 37°C for 24 h, was measured and interpreted as sensitive if the ZOI was ≥ 22 mm and resistant if the ZOI was ≤ 21 mm, as described in CLSI guidelines [24]. *S. aureus* strains resistant to cefoxitin discs were considered methicillin resistant (MRSA). *S. aureus* ATCC 25923 was used as a quality control strain.

DNA extraction from *S. aureus*

DNA extraction from *S. aureus* isolates was performed via the boiling method as described by Hartas et al. [21]. A NanoDrop Spectrophotometer (Thermo Scientific, USA) was used to measure the purity of extracted DNA, and was considered acceptable for PCR amplification if the value of A_{260}/A_{280} was 1.8–2.0.

Detection of the *mec*A gene and virulence genes (*clf*A, *clf*B and *tst*)

Conventional PCR amplification was carried out for *mec*A and virulence genes (*clf*A gene, *clf*B gene and *tst* gene) in the DNA extracted from *S. aureus* isolates. The primers (Sangon Biotech Co. Ltd., China) used are listed in **Table 1**. The PCR mixture was composed of 1x master mix (SolisBiodyne, Estonia), 0.2 µM primers (each of the forward and reverse), 2 µL of DNA template and nuclease-free water to maintain the volume of the reaction mixture at 20 µL. Positive controls prepared as synthetic plasmid constructs containing the nucleotide sequence corresponding to the PCR amplicon between the forward and reverse primer binding sites for each target gene (Sangon Biotech Co. Ltd., China) and a negative control without a DNA template were also used as PCR controls. The PCRs included initial denaturation at 95°C for 5 min; 30 cycles of amplification (denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 40 s) for the *mec*A gene; 35 cycles of amplification (denaturation at 95°C for 15 s, annealing at 56°C for 30 s, and extension at 72°C for 40 s) for the *clf*A gene; 35 cycles of amplification (denaturation at 95°C for 30 s, annealing at 50.8°C for 30 s, and extension at 72°C for 40 s) for the *clf*B gene; and 35 cycles of amplification (denaturation at 95°C for 30 s, annealing at 52°C for 60 s, and extension at 72°C for 40 s) for the *tst* gene; and a final extension at 72°C for 5 min for all four genes. An agarose gel electrophoresis (2% agarose gel with 0.5 µg/mL ethidium bromide) at 100 V for 1 h was performed for the detection of the PCR products and visualized via a UV transilluminator.

Table 1: Sequences of the primers used and their amplicon sizes

Gene of interest	Primer sequence (5′-3′)	Amplicon size (bp)	References
***clf*A**	F: CGCCGGTAA CTGGTGAAGCT R:TGCTCTCATT CTAGGCGCACTT	314	[26]
***clf*B**	F: ATGATCTTG CTTGCGTT R: CCGATTCAA GAGTTACACC	215	[26]
tst	F: ACCCCTGTT CCCTTATCATC R: TTTTCAGTAT TTGTAACGCC	326	[20]
***mec*A**	F: GTAGAAATGA CTGAACGTCCG ATAA R: CCAATTCCACATT GTTTCGGTCTAA	310	[27]

Laboratory quality control

All culture media underwent sterility and performance testing before use [23]. PCR assays included positive and negative controls in each run to ensure assay validity.

Data analysis

Data obtained from phenotypic screening for methicillin resistance and gene detection were entered into MS Excel, and the statistical analysis was carried out in SPSS Version 21. Fisher's exact test was used to determine the associations between the phenotypic and genotypic occurrence of methicillin resistance and the co-occurrence of the *mec*A gene and the virulence genes, and association of semen quality with the methicillin resistance and virulence genes, under 95% confidence interval and 5% standard error. A value of $p < 0.05$ was considered significant.

Results

A semen culture of 217 samples resulted in bacteriospermia in 55 (25.3%) samples. A total of 62 isolates were obtained from 55 samples. Of total 62 isolates, 21 (33.9% of the bacterial isolates) were *S. aureus*.

Methicillin resistance in *S. aureus*

The cefoxitin disc diffusion test revealed phenotypic methicillin resistance in 12 of the 21 *S. aureus* strains (57.14%). All *S. aureus* isolates were also examined for the mecA gene, among which 14 (66.67%) carried the gene. In this study, 4 isolates harboring the *mec*A gene were susceptible to the cefoxitin disc, and the *mec*A gene was not detected in 2 isolates that were resistant to the cefoxitin disc (**Table 2**). Overall concordance between cefoxitin disc diffusion and *mec*A PCR was 71.4%. Discordant results were observed in 28.6% of isolates, including four *mec*A-positive but phenotypically sensitive isolates and two *mec*A-negative but phenotypically resistant isolates. Cefoxitin-resistant isolates had 6.25-fold higher odds of harboring the mecA gene than cefoxitin-sensitive isolates (OR = 6.25, 95% CI: 0.84–46.62). The association between phenotypic and genotypic resistance was not statistically significant (p = 0.159).

Table 2. Phenotypic and genotypic resistance to methicillin in *S. aureus*

Characteristics of *S. aureus* isolates	Resistant to Cefoxitin disc	Sensitive to Cefoxitin disc	Concordance interpretation
*mec*A gene present	10	4	10 true MRSA, 4 discordant
*mec*A gene absent	2	5	5 true MSSA, 2 discordant
Total	12	9	

Status of virulence genes (*clf*A, *clf*B, and *tst* genes)

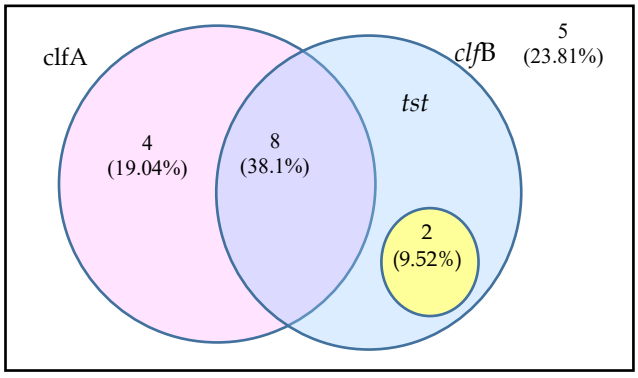

Figure 1: Venn diagram showing the distribution and co-occurrence of the virulence genes clfA, clfB, and tst among *Staphylococcus aureus* isolates (n = 21)

The genes responsible for clumping factor A (*clf*A gene) and clumping factor B (*clf*B gene) were present in 12 (57.14%) isolates, and the gene responsible for toxic shock syndrome (*tst* gene) was present in only 2 (9.52%) isolates of *S. aureus*. Sixteen (76.19%) isolates contained at least one of the two genes, clf*A* or clf*B*, which encode the clumping factor. However, 5 (23.81%) isolates did not contain any clumping factor genes. At least one virulence gene under study was present in 16 (76.19%) isolates (**Figure 1**).

Association of the *mec*A gene and virulence genes under study

The co-occurrence of the *mec*A gene and the virulence genes *clf*A, *clf*B, and *tst* was studied, and the associations were determined via Fisher's exact test. Although more *S. aureus* strains were detected with the *mec*A gene together with the *clf*A and *clf*B genes, there was no significant association between the co-occurrence of the *mec*A gene and each of the virulence genes under study (*p* value >0.05 for all three associations). The odds ratio (OR) with 95% confidence interval (CI) for the occurrence of *mec*A gene with each of the virulence genes under study (*clf*A, *clf*B and *tst* genes) show statistically insignificant associations (**Table 3**).

Table 3: Frequency of detection of the *mec*A gene and virulence genes in *S. aureus* isolates

Genes under study	*clf*A gene detection		*clf*B gene detection		*tst* gene detection	
	Present	Absent	Present	Absent	Present	Absent
*mec*A gene present	9	5	10	4	2	12
*mec*A gene absent	3	4	2	5	0	7
Odds ratio (95% CI)	2.40 (0.42-13.66)		6.25 (0.84-46.62)		3.00 (0.13-70.88)	
p-value	0.397		0.159		0.533	

p-value < 0.05; the relatively small sample size may limit statistical power and generalizability of the findings.

Association of methicillin resistance and virulence genes of *S. aureus* with semen quality

Fisher's exact test and OR with 95% (CI) were used to evaluate the association of methicillin resistance (phenotypic and genotypic) and virulence genes (*clfA*, clfB, and *tst*) with semen quality (based on sperm concentration, motility, morphology and vitality of sperms). No statistically significant associations were observed between semen quality and phenotypic methicillin resistance (OR = 3.75, 95% CI: 0.59–23.94), *mecA* positivity (OR = 0.17, 95% CI: 0.02–1.77), *clfA* positivity (OR = 3.75, 95% CI: 0.59–23.94), *clfB* positivity (OR = 1.60, 95% CI: 0.27–9.49), or *tst* positivity (OR = 0.58, 95% CI: 0.03–10.86) (all Fisher's exact test p > 0.05) (**Table 4**). Although phenotypic methicillin resistance and *clf*A positivity showed higher odds of abnormal semen quality, and *mec*A and *tst* positivity showed lower odds, all 95% confidence intervals included 1, indicating no significant association between methicillin resistance or the investigated virulence genes and semen quality in the study population.

Table 4: Association of methicillin resistance and virulence genes of *S. aureus* with semen quality

Variable	Characterization	Semen quality		Fisher's exact *p*-value	Odds ratio (OR)	95% confidence interval
		Abnormal	Normal			
Phenotypic methicillin resistance	Sensitive	4	5	0.203	3.75	0.59-23.94
	Resistant	9	3			
***mec*A gene**	Absent	6	1	0.174	0.167	0.02-1.77
	Present	7	7			
***clf*A gene**	Absent	4	5	0.203	3.75	0.59-23.94
	Present	9	3			
***clf*B gene**	Absent	5	4	0.673	1.60	0.27-9.49
	Present	8	4			
***tst* gene**	Absent	12	7	1.000	0.58	0.03-10.86
	Present	7	1			

p-value<0.05 is considered as significant.

Discussion

S. aureus, a normal flora of the mucosal membrane of humans and animals, is also a common pathogen of causing various human diseases [28]. The organism is also generally present in the semen of fertile and infertile men [5]. *S. aureus* was the most common bacterium isolated in this study. It has also been frequently isolated from semen in several studies. *S. aureus* accounted for approximately 40% of the total bacteria in semen in various studies from India, Egypt, and Nigeria [1,11,29]. It was also commonly isolated from semen in studies performed in Germany [30]. The presence of *S. aureus* in semen may not always represent seminal infection and is responsible for the decrease in the fertility potential of spermatozoa in men. However, their presence in semen should not be neglected as contamination.

We observed an insignificant difference in the rate of methicillin resistance detected by cefoxitin disc diffusion and *mec*A gene amplification. However, a few of the isolates harbouring the *mec*A gene were resistant to the cefoxitin disc. Like our findings, *mec*A-positive *S. aureus* were found to be susceptible to oxacillin and cefoxitin discs [31]. Although the detection rates of *mec*A and cefoxitin susceptibility are considered highly comparable [32,33], discrepancies in MRSA detection might occur. Theoretically, the resistance of the bacteria towards β-lactam antibiotics is also possible in isolates whose gene expression of the altered protein penicillin-binding protein 2a (PBP2a) is low or absent [34]. Additionally, our study reported a few *S. aureus* strains lacking the *mec*A gene and showing resistance to cefoxitin discs. MRSA strains without the *mec*A gene have also been reported in *S. aureus* isolates from milk samples in different studies [35,36] and in isolates from clinical samples [37], possibly because of heterogeneous expression of *mec*A, regulatory factors influencing gene expression, mutations affecting resistance phenotypes, and the possible involvement of alternative resistance determinants such as *mec*C, a homologue of the *mec*A gene, instead of the *mec*A gene [38,39]. A recent study on clinical MRSA for the detection of *mec*A and *mec*C genes revealed the higher prevalence of *mec*C gene than the *mec*A gene [40]. However, our study did not detect any other gene responsible for methicillin resistance and needs further detailed study. Future investigations incorporating additional molecular targets, including *mec*C, would provide a more comprehensive understanding of resistance mechanisms among seminal *S. aureus* isolates.

S. aureus can infect any part of the body due to the presence of various toxins, enzymes, and other virulence factors that are responsible for the pathogenicity of the bacteria. Fibrinogen-binding proteins, known as clumping factors A and B, are located on the cell surface of *S. aureus* and are virulence factors that play a role in attachment to host cells [41,42]. In our study, we detected at least one of the genes responsible for clumping factor production in more than three-quarters of the isolates. The *clf*A gene was detected in more than half of the isolates. Another prominent virulence gene in our study was the *clf*B gene, which was also present in more than half of the isolates. Similar to our study, 58% of *S. aureus* isolates from dairy farms possessed the *clf*A gene [43]. A similar percentage of *S. aureus* isolates causing bovine mastitis were detected with the *clf*A gene [26]. A study on *S. aureus*-derived vascular abscess infection reported the occurrence of the genes *clf*A and *clf*B in all the isolates, indicating that these genes are significant virulence genes of pathogenic *S. aureus* [44]. Both the *clf*A and *clf*B genes were detected at high frequencies in the urinary pathogen *S. aureus* [19]. The *clf*A gene product clumping

factor A is considered an important virulence factor responsible for the aggregation of protein-coated parts of the host in various infections [45]. The presence of virulence genes in these bacteria may initiate pathogenesis and agglutinate sperm cells, impairing sperm motility.

An understanding of the co-occurrence of virulence and antimicrobial resistance genes in *S. aureus* isolated from semen is important for assessing both pathogenic potential and antibiotic resistance in reproductive tract infections. The co-occurrence of *mec*A and virulence genes in *S. aureus* might further increase the pathogenicity of bacteria. The co-occurrence of clumping factor genes (*clf*A and *clf*B) with *mec*A in MRSA increases the ability of bacteria to adhere to spermatozoa, potentially impairing semen quality. We could not establish a correlation between the *mec*A gene and any of the virulence genes studied, which may be due less sample size. However, studies on clinical *S. aureus* isolates revealed a high prevalence of adhesion genes (*clf*A and *clf*B genes) among MRSA, indicating the coexistence of adhesion genes and *mec*A genes [46].

We also detected another virulence gene, the *tst* gene, which encodes toxic shock syndrome toxin, to understand the virulence property of *S. aureus* present in semen [47]. However, the *tst* gene was detected in only a few *S. aureus* isolates, which might be a reflection of a smaller number of *S. aureus* tested. Future studies incorporating larger sample sizes is recommended. Various other studies on *S. aureus* from different clinical samples also revealed that the *tst* gene is not commonly present as a virulence gene. No *tst* gene was detected in *S. aureus* in previous studies, with clinical samples such as seminal fluid [20], abscess [44], and endocervix [48].

In this study, *S. aureus* isolates harboring the *tst* gene also contained the *mec*A gene. However, no significant co-occurrence was detected between the two genes. A study on the co-occurrence of antibiotic-resistant virulence genes in MRSA detected no *tst* genes in isolates with the *mec*A gene [49]. In another study, both the *tst* gene (68%) and the *mec*A gene (87.3%) were detected in *S. aureus* isolates from clinical samples by Koosha and colleagues; however, there was no significant co-occurrence of these genes [47].

The virulence genes *clf*A, *clf*B, and *tst* were selected because they represent important pathogenic mechanisms of *S. aureus*. These genes were chosen for the assessment of adhesion-related and toxin-mediated virulence characteristics among seminal isolates. Furthermore, *S. aureus* possesses a wide array of virulence determinants that were not investigated in this study. Future studies including additional virulence genes such as *spa* (protein A), *hla* (α-hemolysin), *pvl* (Panton–Valentine leukocidin), and fibronectin-binding proteins (*fnb*A and *fnb*B), are recommended to obtain a more comprehensive understanding of the pathogenic potential of seminal isolates.

The present study investigated whether methicillin resistance and selected virulence determinants of seminal *S. aureus* isolates were associated with semen quality. Although phenotypic methicillin resistance and the presence of the *clfA* gene showed higher odds of abnormal semen quality, whereas *mecA* and *tst* positivity showed lower odds, none of these associations were statistical significance. Likewise, the *clfB* gene was not significantly associated with semen quality. These findings indicate that the presence of methicillin resistance or the investigated virulence genes alone may not be sufficient to explain impaired semen quality in this study population. The lack of significant associations may be attributed to the limited number of *S. aureus* isolates included in the analysis. A major limitation of this study is the relatively small number of *S. aureus* isolates (n = 21), reducing the statistical power and difficulty in drawing definitive conclusions regarding the prevalence and distribution of resistance and virulence genes among seminal isolates. Furthermore, the findings may not be representative of the broader infertile male population in Nepal. Additionally, the cross-sectional observational study design could identify the associations between the virulence genes and semen quality and could not establish causal relationships.

Conclusion

This study demonstrated the substantial occurrence of antimicrobial resistance and virulence-associated *S. aureus* in semen. The study findings highlight the potential value of microbiological screening and antimicrobial susceptibility testing in the investigation of infertile men, particularly those with suspected genital tract infections or unexplained infertility. However, the relatively small number of isolates analysed requires careful interpretations. Studies with larger sample sizes are needed to clarify the clinical significance of these findings in the context of male reproductive health.

Author's Contribution

ASh-Laboratory and Data Analysis, Writing-Original Draft Preparation; DRJ-Conceptualization, Supervision, Writing-Review and Editing; DV-Laboratory analysis;

SMS-Material contribution; ASi-Supervision and Writing-Review and Editing.

Competing interest

No competing interests were disclosed.

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Ethical approval and consent

This study was approved by Nepal Health Research Council (NHRC) (Reg. no.: 874/2019).

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