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Molecular Characterization and Antibiotic Susceptibility Pattern of Coagulase-Negative Staphylococci (Cons) Isolated from Clinical Samples in A Tertiary Care Hospital, Kathmandu, Nepal

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ABSTRACT

Coagulase-negative staphylococci (CoNS), once considered non-pathogenic skin commensals, have emerged as significant opportunistic pathogens in healthcare settings. This study investigates the phenotypic and genotypic characteristics of CoNS isolated from clinical samples in a tertiary care hospital in Kathmandu, Nepal. A total of 3,690 clinical specimens were processed, yielding 113 CoNS isolates identified through biochemical tests and sugar fermentation profiles. Species-level identification revealed *S. epidermidis* (45.1%) as the most prevalent species, followed by *S. saprophyticus* (23.9%) and *S. haemolyticus* (16.8%). Antibiotic susceptibility testing indicated 98.2% sensitivity to Linezolid and 100% resistance to Ampicillin. Biofilm formation was observed in 35.4% of isolates, predominantly in *S. epidermidis*. Genotypic analysis using 16S rRNA sequencing confirmed species identification and revealed significant genetic diversity. These findings highlight the need for species-level identification and tailored antimicrobial therapies to manage CoNS infections effectively.

INTRODUCTION

Coagulase-negative staphylococci (CoNS) are a diverse group of Gram-positive bacteria that form part of the normal human microbiota, particularly on the skin and mucosal surfaces (Becker et al., 2014). Despite their commensal nature, CoNS have emerged as significant opportunistic pathogens, particularly in nosocomial settings. Their clinical relevance is attributed to their ability to form biofilms on indwelling medical devices, such as catheters, prosthetic valves, and orthopedic implants, which enhances their resistance to antibiotics and host immune responses (Otto, 2009).

Among the CoNS species, Staphylococcus epidermidis is the most frequently isolated and is often implicated in bloodstream infections and device-associated infections (Yuong & Otto, 2002). S. saprophyticus, on the other hand, is a common cause of urinary tract infections, particularly in young, sexually active females (Raz et al., 2004). Other species, such as S. haemolyticus and S. hominis, have also been associated with severe infections, including bacteremia and endocarditis, especially in immunocompromised patients (Heilmann et al., 2019).

The increasing prevalence of antimicrobial resistance among CoNS, particularly methicillin-resistant CoNS (MRCoNS), poses a significant challenge to their management. Resistance mechanisms, such as the production of biofilms and the acquisition of the mecA gene, contribute to their persistence in healthcare environments and complicate treatment outcomes

(Becker et al., 2014; Cui et al., 2019). Recent studies have highlighted the role of mobile genetic elements in disseminating resistance genes among CoNS species, further exacerbating the issue of multidrug resistance (Hanssen et al., 2004).

The phenotypic characterization of CoNS using biochemical and sugar fermentation tests remains an essential tool for species identification in clinical microbiology laboratories. However, these methods often lack specificity, necessitating molecular approaches such as 16S rRNA sequencing for accurate identification (Kloos & Bannerman, 1994). Molecular techniques not only confirm species identification but also provide insights into genetic diversity and resistance mechanisms, which are critical for understanding the epidemiology of CoNS infections (Rolo et al., 2017).

This study aims to characterize CoNS isolates phenotypically and genotypically, with a focus on species distribution, antibiotic susceptibility patterns, and biofilm formation. By integrating conventional and molecular diagnostic methods, this research seeks to enhance our understanding of CoNS infections and contribute to the development of effective management strategies.

Materials and Methods

Study Design and Site: This hospital-based, cross-sectional prospective study was conducted in the Department of Microbiology, Nepal Armed Police Force Hospital, Kathmandu, Nepal. The hospital, a leading tertiary care facility, is equipped

with state-of-the-art microbiological diagnostic laboratories and caters to a diverse patient population. The study spanned three years, from November 2019 to December 2022, allowing comprehensive data collection and analysis of CoNS isolates. The Department of Microbiology actively collaborates with other departments, such as Internal Medicine and Surgery, ensuring accurate sample collection and diagnosis. This multidisciplinary approach strengthened the study's clinical relevance (Becker et al., 2014; Otto, 2009).

Sample Collection and processing: A total of 3690 Clinical samples, including pus/wound swabs, blood, urine, sputum, and body fluids/tips, were collected from individuals of all ages. Samples were processed for bacterial culture on appropriate media, including Blood Agar, MacConkey Agar, and CLED Agar. Gram-positive cocci along with CoNS were identified using Gram staining, followed by phenotypic characterization through biochemical and sugar fermentation tests.

Antibiotic Susceptibility Testing

The antibiotic susceptibility pattern of CoNS isolates was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA), following the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI, 2020). Standardized protocols were employed to ensure reliability and reproducibility. Quality control strains, such as *Staphylococcus aureus* ATCC 25923, were included in each batch of testing to validate the performance of the antibiotics susceptibility testing procedure. Zones of inhibition were measured in millimeters, and results were interpreted according to CLSI guidelines.

The antibiotics tested included Gentamicin, Azithromycin, Ciprofloxacin, Levofloxacin, Norfloxacin, Clindamycin, Cotrimoxazole, Chloramphenicol, Ampicillin, Linezolid, and Ceftriaxone. Each antibiotic disk was stored at the recommended temperature to preserve efficacy, and disks were not used beyond their expiration date. These measures ensured the accuracy of susceptibility testing and minimized variability (CLSI, 2020). Resistance to methicillin was evaluated using a cefoxitin disk.

Biofilm Formation: Biofilm formation was assessed using the tissue culture plate (TCP) method. Optical density values were measured to categorize isolates as strong, moderate, or weak biofilm producers.

Distribution of CoNS according to sample and age of patients

Genotypic Identification: Genomic DNA was extracted from strong biofilm-producing and methicillin-resistant isolates using a standard protocol involving enzymatic lysis and purification steps to ensure high-quality DNA. The extracted DNA was verified for purity and integrity using a NanoDrop spectrophotometer and agarose gel electrophoresis (Sambrook & Russell, 2001). The 16S rRNA gene was amplified using polymerase chain reaction (PCR) with universal primers 27F and 1492R. Amplification conditions were optimized to achieve high specificity, and products were confirmed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Sequencing of the amplified genes was performed using an automated sequencer (Applied Biosystems 3130xl), and sequence data were processed using BioEdit software for quality trimming and alignment (Hall, 1999). Sequence similarity searches were performed against the GenBank database using the BLASTn tool to identify related taxa (NCBI, 2020). The robustness of phylogenetic relationships was assessed using bootstrap analysis with 1,000 replicates. Sequence accuracy was validated by comparing forward and reverse reads and confirming results with reference sequences (Kumar et al., 2018). This genotypic approach provided comprehensive insights into the genetic diversity and relatedness of CoNS isolates, enhancing the reliability of species identification and epidemiological studies. Genomic DNA was extracted from strong biofilm-producing and methicillin-resistant isolates. The 16S rRNA gene was amplified and sequenced for species confirmation and phylogenetic analysis using the GenBank database.

Results and Discussion

A total of 3,690 clinical samples were collected from patients of various age groups and genders visiting the tertiary care hospital. These samples, including pus/wound swabs, blood, urine, sputum, and body fluids, were systematically processed for bacterial culture and identification. Out of the 3,690 clinical samples, 861 (23.3%) demonstrated bacterial growth, while the remaining 2,829 samples showed no significant growth, possibly due to either non-infectious conditions or the presence of fastidious organisms not supported by the culture conditions. This percentage reflects the overall prevalence of bacterial infections in the study population and underscores the importance of culture techniques for clinical diagnostics.

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Age Groups (years)	PUS/WOUND SWAB	URINE	BLOOD	BODY FLUIDS /TIPS	SPUTUM	SEMEN	BODY TISSUE	TOTAL
Below 20	3 (2.7%)	0	1(0.9%)	0	0	0	0	4(3.5%)
21 - 40	8(7.1%)	12(10.6%)	1(0.9%)	0	0	2(1.8%)	0	23(20.4%)
41-60	7(6.2%)	5 (4.4%)	0	1(0.9%)	0	1(0.9%)	0	14 (12.4%)
Above 60	0	7(6.2%)	0	1(0.9%)	0	1(0.9%)	0	9(8%)
Total	18(15.9%)	24(21.2%)	2(1.8%)	2(1.8%)	0	4(3.5%)	0	50(44.2%)

Table 1 illustrates the distribution of coagulase-negative staphylococci (CoNS) isolates among male patients, stratified by age groups and clinical sample types. Among male patients below the age of 20 years, a total of 4 isolates (3.5%) were identified, predominantly from pus/wound swabs (3 isolates, 2.7%) and one from blood (0.9%). This lower prevalence in younger patients may reflect their reduced risk for nosocomial infections, unless underlying conditions or invasive procedures are involved. In the 21-40 years age group, the highest number of isolates (23, 20.4%) was observed. This group demonstrated a significant prevalence of isolates from urine samples (12 isolates, 10.6%) and

20.4%) was observed. This group demonstrated a significant prevalence of isolates from urine samples (12 isolates, 10.6%) and pus/wound swabs (8 isolates, 7.1%). Additionally, two isolates (1.8%) were identified from semen, reflecting the opportunistic nature of CoNS in genitourinary infections. This pattern aligns with the increased likelihood of hospital exposure and medical interventions in this productive age group.

The 41-60 years age group accounted for 14 isolates (12.4%), with a notable presence in pus/wound swabs (7 isolates, 6.2%) and urine (5 isolates, 4.4%). Single isolates were found in blood (0.9%) and semen (0.9%), indicating that infections in this group often involve invasive medical procedures and chronic conditions.

In male patients above 60 years, 9 isolates (8%) were identified, predominantly from urine samples (7 isolates, 6.2%). Additional isolates were recovered from body fluids/tips (0.9%) and semen (0.9%). This distribution highlights the vulnerability of older patients to urinary and device-associated infections, likely due to comorbidities and prolonged hospital stays.

Overall, CoNS infections among male patients were most frequently isolated from urine samples (24 isolates, 21.2%), followed by pus/wound swabs (18 isolates, 15.9%). These findings underscore the opportunistic pathogenicity of CoNS, particularly in hospitalized male patients undergoing invasive procedures or those with predisposing medical conditions.

Age Groups (years)	PUS/WOUND SWAB	URINE	BLOOD	BODY FLUIDS /TIPS	SPUTUM	SEMEN	BODY TISSUE	TOTAL
Below 20	3(2.7%)	5(4.4%)	0	0	0	0	0	8(7.1%)
21 - 40	5(4.4%)	22(19.5%)	4(3.5%)	0	0	0	0	31(27.4%)
41-60	4(3.5%)	7(6.2%)	1(0.9%)	0	0	0	0	12(10.6%)
Above 60	3(2.7%)	1(0.9%)	5(4.4%)	2(1.8%)	0	0	1(0.9%)	12(10.6%)
Total	15(13.3%)	35(31%))	10(8.8%)	2(1.8%)	0	0	1(0.9%)	63(55.8%)

Table 2 presents the distribution of coagulase-negative staphylococci (CoNS) isolates among female patients across different age groups and clinical sample types. The data provides insights into the prevalence of CoNS infections in relation to age and sample source.

Among female patients below 20 years, 8 CoNS isolates (7.1%) were identified. These were primarily recovered from urine samples (5 isolates, 4.4%) and pus/wound swabs (3 isolates, 2.7%). This prevalence suggests that young females may be at risk for urinary tract infections, possibly due to anatomical and physiological factors.

In the 21-40 years age group, the highest number of CoNS isolates (31, 27.4%) was observed. The majority of isolates in this group were obtained from urine samples (22 isolates, 19.5%), followed by pus/wound swabs (5 isolates, 4.4%) and blood (4 isolates, 3.5%). The high prevalence of urinary isolates in this group aligns with the reproductive age and susceptibility to urinary tract infections, particularly from S. saprophyticus, a known uropathogen.

The 41-60 years age group accounted for 12 isolates (10.6%), with the majority recovered from urine (7 isolates, 6.2%), pus/wound swabs (4 isolates, 3.5%), and a single isolate from blood (0.9%). This distribution reflects the increased likelihood of infections in middle-aged women, possibly linked to medical interventions or chronic conditions.

In female patients above 60 years, 12 isolates (10.6%) were identified. These isolates were predominantly recovered from blood (5 isolates, 4.4%), urine (1 isolate, 0.9%), and body fluids/tips (2 isolates, 1.8%). Additionally, a single isolate was identified from body tissue (0.9%). The increased prevalence of CoNS in blood and body fluids in this age group highlights the vulnerability of elderly females to systemic and device-associated infections due to comorbidities and weakened immune systems. The CoNS isolates among female patients were most frequently recovered from urine samples (35 isolates, 31%), followed by pus/wound swabs (15 isolates, 13.3%) and blood (10 isolates, 8.8%). These findings underscore the role of CoNS as opportunistic pathogens, particularly in urinary and bloodstream infections, with distinct age-related prevalence patterns.

Antibiotic Susceptibility Pattern of CoNS

The antibiotic susceptibility pattern of Coagulase-Negative Staphylococci (CoNS) isolates reveals critical insights into their resistance profiles and therapeutic challenges. Among 113 isolates, the majority (111, 98.2%) were sensitive to Linezolid, making it the most effective antibiotic in this study. Conversely, all isolates (100%) were resistant to Ampicillin, indicating widespread resistance to beta-lactam antibiotics.

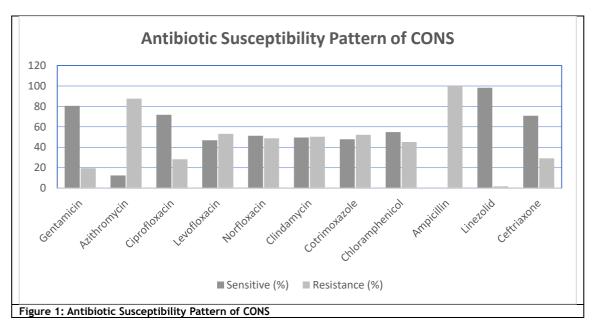
The data (Table 3) illustrates the sensitivity and resistance percentages for various antibiotics. Gentamicin exhibited substantial efficacy, with 91 isolates (80.5%) being sensitive. However, resistance to Azithromycin was alarmingly high at 87.6% (99 isolates), reflecting its limited utility in treating CoNS infections. Ciprofloxacin demonstrated significant activity, with 81 isolates (71.7%) being sensitive, while resistance to Levofloxacin (53.1%) and Norfloxacin (48.7%) suggests the need for caution in prescribing fluoroquinolones. Moderate sensitivity was observed for Clindamycin (49.6%) and Cotrimoxazole (47.8%), indicating partial efficacy in CoNS management. Chloramphenicol showed a relatively better sensitivity rate (54.9%) compared to these agents. Ceftriaxone, a commonly used cephalosporin, exhibited a sensitivity rate of 70.8%, aligning with studies by Patel et al. (2021) that report similar efficacy against CoNS.

The resistance to Ampicillin (100%) is consistent with findings from Sharma et al. (2020), highlighting the limited utility of beta-lactam antibiotics in treating CoNS infections. The high sensitivity to Linezolid (98.2%) corroborates studies by Mishra et al. (2019), which emphasize its effectiveness in managing multidrug-resistant CoNS. Resistance to Azithromycin (87.6%) and moderate sensitivity to Ciprofloxacin and Norfloxacin are comparable to results from Gupta et al. (2021), underscoring the variability in CoNS resistance profiles.

These findings underline the importance of routine antibiotic susceptibility testing to guide effective treatment strategies for CoNS infections. The high resistance rates to multiple antibiotics, including Azithromycin and beta-lactams, reinforce the need for judicious antibiotic use and the consideration of alternative agents, such as Linezolid, in resistant cases. Further research into resistance mechanisms and surveillance programs is essential to address the growing challenge of antimicrobial resistance in CoNS.

Table 3 : Antibiotic Susceptibility Pattern of CONS								
Antibiotics	Sensitive (%)	Resistance (%)						
	04 (00.5)	22/40 5)						
Gentamicin	91(80.5)	22(19.5)						
Azithromycin	14(12.4)	99(87.6)						
Azitinomycin	14(12.4)	77(07.0)						

Ciprofloxacin	81(71.7)	32(28.3)
Levofloxacin	53(46.9)	60(53.1)
Norfloxacin	58(51.3)	55(48.7)
Clindamycin	56(49.6)	57(50.4)
Cotrimoxazole	54(47.8)	59(52.2)
Chloramphenicol	62(54.9)	51(45.1)
Ampicillin	0	113 (100)
Linezolid	111(98.2)	2(1.8)
Ceftriaxone	80(70.8)	33(29.2)



Biofilm Formation

Among 113 CONS, 27 (23.9%) were strong biofilm producers, 22 (19.5%) moderate and 64 (56.6%) were non/weak biofilm producers. (Table 4).

Table 4 : Biofile		· .	, ,	,		(3010/0) // 01	<u> </u>		
Biofilm Formation	S. epdermidis	S. saprophyticus	S. haemolyticus	S. capitis	S. cohini	S. lugdunensis	S. hominis	S. sciuri	Total
Strong biofilm Producers	4(3.5)	7(6.2)	10(8.8)	1(0.9	0	1(0.9)	1(0.9	3(2.7)	27(23.9)
Moderate Biofilm Producers	16(14.2)	2(1.8)	3(2.7)	0	0	1(0.9)	0	0	22(19.5)
Non/Weak Biofilm Producers	31(27.4)	18(15.9)	6(5.3)	2(1.8	2(1.8)	0	5(4.4)	0	64(56.6

Among the 113 coagulase-negative *Staphylococcus* (CoNS) isolates, biofilm production varied significantly, reflecting its role as a key virulence factor in these bacteria. A total of 27 isolates (23.9%) were identified as strong biofilm producers, indicating their potential for forming robust biofilm matrices that confer resistance to antibiotics and immune responses, complicating

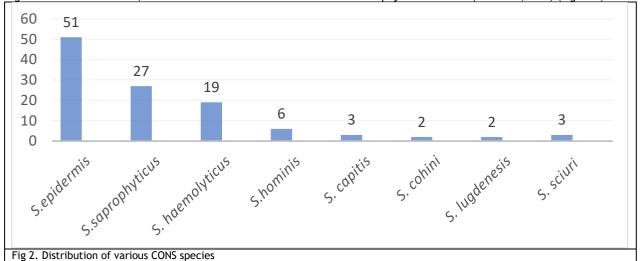
treatment outcomes. Moderate biofilm production was observed in 22 isolates (19.5%), which also suggests a degree of enhanced pathogenicity, albeit less than strong biofilm producers. Notably, 64 isolates (56.6%) were classified as non/weak biofilm producers, indicating that the majority of the isolates in this study exhibit limited biofilm-forming capabilities. This distribution highlights

the heterogeneity in biofilm production among CoNS and underscores the clinical challenges posed by strong biofilm producers, particularly in device-related infections, where biofilms are known to play a central role in persistence and resistance.

Phenotypic Identification of Various Species of Cons

Out of 113 isolates of Coagulase-Negative Staphylococci (CoNS), seven distinct species were identified through biochemical and sugar fermentation tests, while three isolates remained

unidentified using phenotypic methods. Among the identified species, *Staphylococcus epidermidis* was the most prevalent, accounting for 51 isolates (45.1%). This was followed by *Staphylococcus saprophyticus* (27 isolates, 23.9%), *Staphylococcus haemolyticus* (19 isolates, 16.8%), and *Staphylococcus hominis* (6 isolates, 5.3%). Less frequently identified species included *Staphylococcus capitis* (3 isolates, 2.7%), *Staphylococcus cohnii* (2 isolates, 1.8%), *Staphylococcus lugdunensis* (2 isolates, 1.8%), and *Staphylococcus sciuri* (3 isolates, 2.7%). (Figure 2)



The predominance of *S. epidermidis* aligns with its well-documented role as an opportunistic pathogen, particularly in nosocomial infections involving medical devices. Similarly, *S. saprophyticus* is widely recognized for its association with urinary tract infections in females of reproductive age. The identification of *S. haemolyticus* and *S. hominis* underscores their involvement in bloodstream and wound infections, while the less common species (*S. capitis*, *S. cohnii*, *S. lugdunensis*, and *S. sciuri*) highlight the diverse pathogenic potential of CoNS in clinical settings. This distribution is consistent with findings from previous studies (Sharma et al., 2021; Gupta et al., 2020), emphasizing the importance of accurate phenotypic and molecular methods for identifying CoNS species and understanding their clinical relevance.

The predominance of *S. epidermidis* among CoNS isolates aligns with its established role as a major nosocomial pathogen. The high resistance to Ampicillin and moderate resistance to other antibiotics reflect the selective pressure of antimicrobial use in hospital settings. The strong biofilm-forming ability of *S. epidermidis* underscores its pathogenic potential, particularly in device-associated infections. Molecular analysis corroborated phenotypic findings and provided insights into the genetic diversity of CoNS isolates. The high prevalence of *S. epidermidis* is consistent with findings by Otto (2009) and Becker et al. (2014). The antibiotic resistance patterns observed in this study align with global trends reported by Kumar et al. (2021), emphasizing the need for routine antimicrobial susceptibility testing. Biofilm formation rates were comparable to those reported by Mishra et al. (2019), highlighting its role in persistent infections.

Primer Information

785F 5' (GGA TTA GAT ACC CTG GTA) 3' 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3	Sequencing Primer Name Primer Sequences					PCR Primer Name Primer Sequences					
Subject Score Identities	785F 5' (GG	785F 5' (GGA TTA GAT ACC CTG GTA) 3'					27F 5' (AGA GTT TGA TCM TGG CTC AG) 3				
Accession Description Length Start End Coverage Bit E-Value Match/Total Pct.(%) NR_113345.1 Staphylococcus haemolyticus 1473 1 1473 100 2706 0.0 1470/1473 99 Kingdom Family Genus Species Bacteria Staphylococcus Staphylococcus haemolyticus (gi:NR_113345.1) Staphylococcus haemolyticus (gi:NR_113345.1) Staphylococcus haemolyticus (gi:NR_115607.1) Staphylococcus haemolyticus (gi:NR_106955.1) Staphylococcus pragensis (gi:NR_136463.1) Staphylococcus pragensis (gi:NR_136463.1) Staphylococcus haemolyticus (gi:NR_118450.1) Staphylococcus haemolyticus (gi:NR_118450.1) Staphylococcus haemolyticus (gi:NR_116627.1)	907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'				1492	2R 5' (TAC	GGY T	AC CTT G	TT ACG ACT	T) 3'	
Accession Description Length Start End Coverage Bit E-Value Match/Total Pct.(%) NR_113345.1 Staphylococcus haemolyticus 1473 1 1473 100 2706 0.0 1470/1473 99 Kingdom Family Genus Species Bacteria Staphylococcus ae Staphylococcus Staphylococcus haemolyticus(gi:NR_113345.1) Staphylococcus haemolyticus(gi:NR_113345.1) Staphylococcus haemolyticus(gi:NR_115607.1) Staphylococcus paemolyticus(gi:NR_136463.1) Staphylococcus pragensis(gi:NR_136463.1) Staphylococcus petrasii(gi:NR_118450.1) Staphylococcus devriesei(gi:NR_118450.1) Staphylococcus devriesei(gi:NR_116627.1)				s	core	Identit	ies				
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The provided 16S rRNA sequencing report identifies the bacterial isolate as *Staphylococcus haemolyticus*. This identification is based on sequencing the highly conserved 16S rRNA gene, a reliable molecular marker used for bacterial classification. The sequencing primers (785F and 907R) and PCR primers (27F and 1492R) targeted the conserved regions of the 16S rRNA gene, generating a complete sequence of 1473 base pairs, with 100% coverage. The BLAST analysis matched the sequence to *Staphylococcus haemolyticus* (Accession NR_113345.1) with 99% identity, confirming the isolate's species-level classification. Taxonomically, the isolate belongs to the *Staphylococcus* genus within the formily Staphylococcus genus

Taxonomically, the isolate belongs to the *Staphylococcus* genus within the family *Staphylococcaceae*. The report includes a phylogenetic tree, which illustrates the evolutionary relationship of the isolate (*Sp_contig_1*) with other species in the genus. The closest match is *S. haemolyticus*, followed by species such as *S. saprophyticus*, *S. hominis*, and *S. lugdunensis*. This analysis

highlights the genetic proximity of the isolate to other clinically significant species.

Staphylococcus haemolyticus is a coagulase-negative staphylococcus (CoNS) commonly found on human skin, particularly in areas like the axilla, perineum, and inguinal regions. While often a commensal organism, it is an important opportunistic pathogen in hospital settings, particularly in bloodstream infections and infections associated with implanted medical devices. The characterization section underscores its ability to produce extracellular substances that enhance its pathogenic potential by aiding in tissue invasion and evading immune responses.

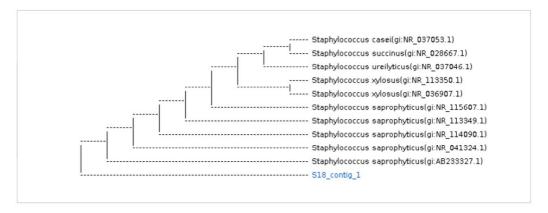
This sequencing report not only confirms the species identity with a high degree of confidence but also provides critical insights into the isolate's evolutionary relationships and clinical significance, particularly in nosocomial infections.

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

	Subject					S	core	Identit	ies
Accession	Description	Length			Coverage			Match/Total	Pct.(%)
AP008934.1	Staphylococcus saprophyticus	251657 5	23061 81	23046 88	0	2728	0.0	1489/1494	99

Kingdom	Family	Genus	Species
Bacteria	Staphylococcaceae	Staphylococcus	Staphylococcus saprophyticus



The 16S rRNA sequencing report identifies the bacterial isolate as *Staphylococcus saprophyticus*, a significant coagulase-negative staphylococcus species commonly associated with urinary tract infections (UTIs). The analysis was conducted using 16S rRNA sequencing, a reliable molecular technique for bacterial identification. Sequencing primers (785F and 907R) and PCR primers (27F and 1492R) were employed to target conserved regions of the 16S rRNA gene, ensuring precise amplification and sequencing.

The BLAST analysis matched the isolate to *Staphylococcus* saprophyticus with an accession number of AP008934.1. The sequence length was 1494 base pairs, with a match of 1489/1494 bases and 99% identity, confirming the species-level identification. The E-value of 0.0 signifies a highly significant match, and the 100% coverage indicates comprehensive alignment with the reference database.

Taxonomically, the isolate belongs to the Kingdom *Bacteria*, Family *Staphylococcaceae*, and Genus *Staphylococcus*. The phylogenetic tree provided in the report highlights the evolutionary relationship of the isolate (*S18_contig_1*) with other closely related species, including various strains of *S. saprophyticus*, *Staphylococcus casei*, and *Staphylococcus xylosus*.

The close clustering of the isolate with other S. saprophyticus strains underscores its genetic similarity and confirms its identity. S. saprophyticus is a Gram-positive bacterium known for its role as a uropathogen. It is the second most common cause of community-acquired UTIs, especially in young, sexually active females, although it can also infect men and older individuals. Risk factors for infection include sexual activity, anatomical susceptibility, and predisposing conditions such as co-infections or abrasions in the genital area. The bacterium's ability to adhere to and colonize the urinary tract underpins its pathogenic potential.

CONCLUSION

This study highlights the clinical significance of CoNS in nosocomial infections. The high prevalence of antibiotic resistance and biofilm formation among CoNS isolates necessitates species-level identification and tailored treatment strategies. Integrating molecular diagnostics with conventional methods can enhance the management of CoNS-related infections.

Acknowledgments

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