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Original Article

Coagulase-Negative Staphylococci as Emerging Nosocomial Pathogens: Antimicrobial Resistance, Methicillin Resistance, and Biofilm Production in a Tertiary Care Hospital

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ABSTRACT

Introduction: Coagulase Negative Staphylococci (CoNS), once considered contaminants, have emerged as important opportunistic pathogens associated with nosocomial infections. Their ability to develop antimicrobial resistance, particularly methicillin resistance, along with biofilm formation, contributes significantly to persistent and device-related infections. Aim of the study was to identify CoNS from various clinical samples, determine their antimicrobial susceptibility pattern, detect methicillin resistance, and evaluate biofilm production.

Material and Methods: A cross-sectional study was conducted in the Department of Microbiology, Mamata Medical College, Khammam, over 18 months. A total of 100 CoNS isolates obtained from clinical specimens were included. Identification was done using standard microbiological techniques. Antimicrobial susceptibility testing was performed by Kirby–Bauer disc diffusion method as per CLSI guidelines. Methicillin resistance was detected using cefoxitin disc diffusion method. Biofilm production was assessed by Congo Red Agar method. Statistical analysis was performed using SPSS, and associations were evaluated using Chi-square test with $p<0.05$ considered significant.

Results: Out of 100 isolates, 67% were from females and the majority belonged to the 21–30 years age group. Urine was the most common sample (56%). High resistance was observed to penicillin (100%), cotrimoxazole (59%), and ciprofloxacin (53%), while linezolid (92%) and doxycycline (79%) showed high sensitivity. Methicillin resistance was detected in 72% of isolates. Biofilm production was observed in 34% of isolates. A significant association was found between methicillin resistance and biofilm production ($p=0.009$). Biofilm production also showed significant association with clinical samples ($p=0.001$).

Conclusion: CoNS are important pathogens with high prevalence of methicillin resistance and multidrug resistance. Biofilm production significantly contributes to antimicrobial resistance and persistence of infection. Routine detection of methicillin resistance and biofilm formation is essential for effective management and infection control.

Keywords: *Coagulase Negative Staphylococci; Methicillin resistance; MRCoNS; Biofilm; Antimicrobial susceptibility; Nosocomial infections.*

INTRODUCTION

Coagulase Negative Staphylococci (CoNS) are Gram-positive, catalase-positive, non-motile cocci belonging to the family Micrococcaceae and genus *Staphylococcus*, typically arranged in clusters (1). These organisms are commensals of human skin and mucosal surfaces, including the anterior nares, external auditory canal, and genitourinary tract, and were

historically regarded as non-pathogenic contaminants in clinical specimens (2). However, over the past few decades, there has been a paradigm shift in their clinical significance, with CoNS emerging as important opportunistic pathogens, particularly in hospital settings (3). The increasing use of indwelling medical devices such as intravascular catheters, prosthetic implants, and other life-support systems, along with a growing population of immunocompromised patients, has contributed significantly to the rise of CoNS-associated infections (4).

CoNS are now recognized as major etiological agents of nosocomial bloodstream infections, accounting for a substantial proportion of hospital-acquired infections worldwide (5). Several studies have documented their role in severe infections among vulnerable populations, including neonates in intensive care units, patients undergoing chemotherapy or organ transplantation, and individuals with prolonged hospitalization (6–8). High-risk groups include patients with indwelling devices, post-operative wounds, and those receiving immunosuppressive therapy (9). Among CoNS species, *Staphylococcus epidermidis* is the most frequently isolated pathogen, responsible for approximately 50–80% of infections, primarily due to its ability to colonize medical devices and form biofilms (10,11). Other species such as *Staphylococcus saprophyticus* are notable causes of urinary tract infections, particularly in sexually active young women (12).

The pathogenicity of CoNS is largely attributed to their ability to produce virulence factors such as extracellular enzymes, hemagglutinins, and notably biofilm formation (13). Biofilm production, mediated by polysaccharide intercellular adhesin (PIA), facilitates adherence to surfaces and protects bacteria from host immune responses and antimicrobial agents (14). Studies have demonstrated that biofilm-forming CoNS isolates are associated with persistent infections and increased resistance to antibiotics due to limited drug penetration, altered metabolic activity, and expression of resistance genes (15). Furthermore, slime production has been correlated with clinically significant bloodstream infections, highlighting its role in pathogenicity (16).

Antimicrobial resistance among CoNS has become a major concern in recent years. Methicillin-resistant CoNS (MRCoNS) constitute a significant proportion of clinical isolates, with reported prevalence rates ranging from 80–90% in hospital settings (17). These strains often exhibit multidrug resistance, including resistance to β-lactams, macrolides, and aminoglycosides, thereby limiting therapeutic options and complicating clinical management (18). The emergence of multidrug-resistant CoNS underscores the need for continuous surveillance of their antimicrobial susceptibility patterns.

Despite increasing recognition of CoNS as significant pathogens, distinguishing true infection from contamination remains a diagnostic challenge. Moreover, there is variability in the prevalence of different CoNS species, biofilm production, and resistance patterns across geographical regions and healthcare settings. Limited data are available from many tertiary care centers regarding the correlation between biofilm formation and methicillin resistance among CoNS isolates, as well as their current antimicrobial susceptibility trends.

Therefore, the present study was undertaken to identify CoNS from various clinical specimens, determine their antibiotic susceptibility patterns, assess methicillin resistance, and evaluate biofilm production as a key virulence factor.

MATERIALS AND METHODS

Study Setting and Design

The present study was conducted in the Department of Microbiology, Mamata Medical College, Khammam. It was designed as a cross-sectional study carried out over a period of 18 months from October 2016 to April 2018. A total of 100 Coagulase Negative Staphylococci (CoNS) isolates obtained from various clinical samples were included in the study

Inclusion Criteria

- Isolates obtained from clinically significant samples such as pus, sputum, urine, blood, body fluids, ear discharge, and throat swabs.
- Samples from patients presenting with symptomatic infections.
- Isolates showing pure growth from infection sites indicating clinical relevance.

Exclusion Criteria

- Isolates considered as contaminants.
- Mixed growth cultures with no clinical significance were excluded from the study.

Study Tools and Data Collection

- Clinical specimens were collected aseptically from patients with suspected infections.
- Relevant clinical details such as type of specimen, patient symptoms, and source of infection were recorded in a structured proforma.
- All isolates were processed in the microbiology laboratory following standard microbiological techniques.

- Laboratory findings including identification, antibiotic susceptibility, methicillin resistance, and biofilm production were systematically documented.

METHODOLOGY

Microscopic Examination

All clinical samples were subjected to Gram staining. Samples showing Gram-positive cocci arranged in clusters were considered suggestive of Staphylococcus species and processed further.

Culture and Identification

Specimens were inoculated onto nutrient agar, blood agar, and mannitol salt agar and incubated at 37°C for 24 hours. Pure isolates were confirmed by repeat Gram staining. Identification of Staphylococcus species was performed using standard biochemical tests including catalase test, modified oxidase test, and coagulase test. Differentiation from Micrococcus species was carried out using Bacitracin (0.04 U) and Furazolidone (100 µg) discs.

Biochemical Tests

- Catalase Test: Performed using 3% hydrogen peroxide. Immediate effervescence indicated a positive result.
- Modified Oxidase Test: Used to differentiate Staphylococcus (negative) from Micrococcus (positive).
- Coagulase Test: Both slide and tube coagulase tests were performed. Isolates negative for both were identified as CoNS.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines. The antibiotics tested included penicillin, erythromycin, clindamycin, cotrimoxazole, ciprofloxacin, doxycycline, linezolid, gentamicin, and nitrofurantoin (for urinary isolates). Quality control was ensured using Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis ATCC 12228

Detection of Inducible Clindamycin Resistance (D-Test)

Inducible clindamycin resistance was detected using the D-test by placing erythromycin and clindamycin discs in proximity. Flattening of the clindamycin zone (D-shaped zone) adjacent to erythromycin indicated inducible resistance.

Detection of Methicillin Resistance

Methicillin resistance among CoNS isolates was determined using the cefoxitin (30 µg) disc diffusion method as per CLSI 2018 guidelines. A zone diameter of ≤24 mm was considered methicillin-resistant, while ≥25 mm was considered sensitive

Detection of Biofilm Production

Biofilm production was assessed using the Congo Red Agar method. Isolates were inoculated onto Congo red agar plates and incubated at 37°C for 24–48 hours. Black colonies with dry crystalline appearance were considered biofilm producers, while red colonies were considered non-producers.

Statistical Analysis

The collected data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) software. Descriptive statistics such as frequency, percentage, mean, and standard deviation were used to summarize the data. Associations between categorical variables such as methicillin resistance and biofilm production were analyzed using the Chi-square test or Fisher's exact test wherever applicable. A p-value of less than 0.05 was considered statistically significant.

RESULTS:

TABLE 1: Demographic Distribution of CoNS Isolates (n=100)

Variable	Category	Number	Percentage (%)
Sex	Male	33	33
	Female	67	67
Age (yrs)	0–10	7	7
	11–20	11	11
	21–30	32	32
	31–40	14	14
	41–50	10	10
	51–60	14	14
	61–70	9	9
	71–80	2	2

	81–90	1	1
Total	—	100	100

The demographic distribution of CoNS isolates showed a clear female predominance, with 67% of isolates obtained from females compared to 33% from males. Age-wise distribution revealed that the majority of isolates were observed in the 21–30 years age group (32%), followed by the 31–40 years and 51–60 years groups (14% each). Lower proportions were noted in the extremes of age, particularly in patients above 70 years (3%). Overall, CoNS infections were most commonly seen in young adults, with a gradual decline in older age groups.

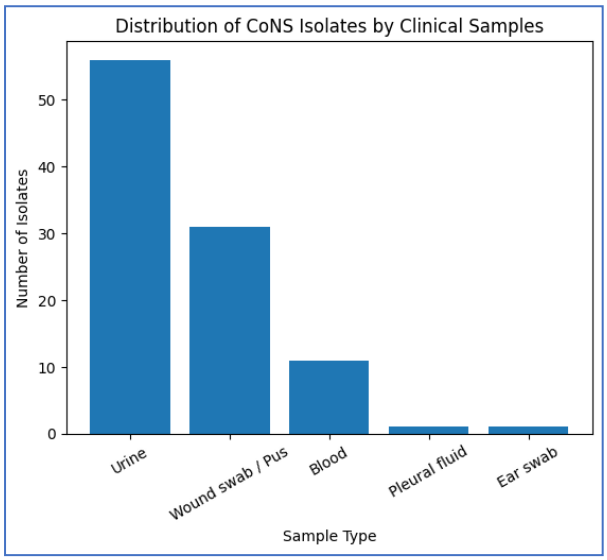

Figure 1: Distribution of CoNS Isolates by Clinical Samples

The figure 1 demonstrates that the majority of CoNS isolates were obtained from urine samples (56%), indicating urinary tract infections as the most common source. This was followed by wound swab/pus samples (31%), reflecting the role of CoNS in skin and soft tissue infections. Blood samples accounted for 11% of isolates, suggesting their involvement in bloodstream infections. Very few isolates were obtained from pleural fluid and ear swab (1% each). Overall, the findings highlight urine as the predominant source of CoNS, with relatively lower occurrence in invasive and other clinical specimens.

TABLE 2: Overall Antibiotic Susceptibility Pattern of CoNS (n=100)

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Nitrofurantoin	44 (44)	6 (6)	6 (6)
Penicillin	0 (0)	0 (0)	100 (100)
Cotrimoxazole	38 (38)	3 (3)	59 (59)
Doxycycline	79 (79)	2 (2)	19 (19)
Ciprofloxacin	43 (43)	4 (4)	53 (53)
Erythromycin	13 (13)	0 (0)	32 (32)
Gentamicin	56 (56)	19 (19)	25 (25)
Linezolid	92 (92)	0 (0)	8 (8)

Inducible Clindamycin Resistance (D-test): 11% (ICR+), 12% (ICR−)

The antibiotic susceptibility pattern of CoNS isolates demonstrated a high level of resistance to commonly used antibiotics. All isolates were resistant to penicillin (100%), indicating widespread β-lactam resistance. High resistance was also observed to cotrimoxazole (59%) and ciprofloxacin (53%), while erythromycin showed moderate resistance (32%). In contrast, linezolid exhibited the highest sensitivity (92%), followed by doxycycline (79%) and gentamicin (56%), suggesting their effectiveness against CoNS infections. Nitrofurantoin showed moderate sensitivity (44%), particularly relevant for urinary isolates. Additionally, inducible clindamycin resistance was detected in a notable proportion of isolates, with 11% showing positive D-test (ICR+), highlighting the importance of routine testing to avoid therapeutic failure.

TABLE 3: Methicillin Resistance Distribution and Sample-wise Association

Variable	MRCoNS n (%)	MSCoNS n (%)	Total	p-value
Urine	44 (78.6)	12 (21.4)	56	
Wound swab/pus	21 (67.7)	10 (32.3)	31	
Blood	6 (54.5)	5 (45.5)	11	

Pleural fluid	1 (100)	0 (0)	1	
Ear swab	0 (0)	1 (100)	1	
Total	72 (72)	28 (28)	100	>0.05

The distribution of methicillin resistance among CoNS isolates showed that a majority were methicillin-resistant (MRCoNS) accounting for 72%, while 28% were methicillin-sensitive (MSCoNS). Among different clinical samples, the highest proportion of MRCoNS was observed in urine samples (78.6%), followed by wound swab/pus (67.7%) and blood (54.5%). Pleural fluid showed 100% methicillin resistance, although the sample size was minimal, while the ear swab isolate was methicillin-sensitive. Despite variation across sample types, the association between clinical samples and methicillin resistance was not statistically significant ($p > 0.05$), indicating that methicillin resistance is widely distributed across different infection sites.

TABLE 4: Antibiotic Susceptibility Pattern of MRCoNS (n=72)

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Nitrofurantoin	33 (45.8)	5 (6.9)	6 (8.3)
Cotrimoxazole	24 (33.3)	2 (2.8)	46 (63.9)
Doxycycline	55 (76.4)	1 (1.4)	16 (22.2)
Ciprofloxacin	25 (34.7)	3 (4.2)	44 (61.1)
Gentamicin	43 (59.7)	15 (20.8)	14 (19.5)
Linezolid	65 (90.3)	—	7 (9.7)
Erythromycin	4 (5.6)	—	25 (34.7)
Clindamycin	12 (16.7)	—	17 (23.6)

The antibiotic susceptibility profile of MRCoNS isolates demonstrated a high degree of multidrug resistance. Maximum resistance was observed to cotrimoxazole (63.9%) and ciprofloxacin (61.1%), indicating limited utility of these commonly used antibiotics. Erythromycin also showed considerable resistance (34.7%), while clindamycin exhibited moderate resistance (23.6%). In contrast, linezolid showed the highest sensitivity (90.3%), followed by doxycycline (76.4%) and gentamicin (59.7%), suggesting their effectiveness against MRCoNS infections. Nitrofurantoin demonstrated moderate sensitivity (45.8%), particularly relevant for urinary isolates. Overall, the findings highlight the significant resistance burden among MRCoNS, emphasizing the need for judicious antibiotic use and routine susceptibility testing.

TABLE 5: Association of Biofilm Production with Methicillin Resistance and Clinical Samples

Variable	Category	Biofilm Present	Biofilm Absent	Total	Odds Ratio	p-value
Methicillin Resistance	MRCoNS	30	42	72	4.28	0.009*
	MSCoNS	4	24	28	—	—
Sample Type	Urine	25	19	44		
	Wound	2	19	21		
	Blood	2	4	6		
	Pleural fluid	1	0	1	6.05	0.001*

*Statistically significant ($p < 0.05$)

The study demonstrated that biofilm production was present in 34% of CoNS isolates. A significant association was observed between methicillin resistance and biofilm production, with MRCoNS showing a higher proportion of biofilm producers (30/72) compared to MSCoNS (4/28). This association was statistically significant ($p = 0.009$) with an odds ratio of 4.28, indicating that methicillin-resistant isolates were more likely to produce biofilms.

Analysis across clinical samples revealed that biofilm production was most common in urine isolates (25 cases), followed by wound samples (2 cases) and blood (2 cases). Pleural fluid showed biofilm production in the single isolate studied. The association between biofilm production and sample type was also statistically significant ($p = 0.001$) with an odds ratio of 6.05. These findings highlight the strong correlation between biofilm formation and methicillin resistance, as well as its role in persistence of infection across different clinical specimens.

DISCUSSION

Coagulase Negative Staphylococci (CoNS), once regarded as mere commensals and contaminants, have now emerged as significant opportunistic pathogens, particularly in hospital settings. The present study evaluated the epidemiological distribution, antimicrobial susceptibility, methicillin resistance, and biofilm production among CoNS isolates from various clinical samples. The findings highlight the increasing clinical importance of CoNS and their evolving resistance patterns. In the present study, a higher proportion of isolates were obtained from females (67%) compared to males (33%). This female predominance may be attributed to the higher number of urinary isolates, as urinary tract infections (UTIs) are more common in females due to anatomical predisposition. Similar findings were reported by Chavan et al. (2017), who observed a higher prevalence of CoNS infections among females, particularly in urinary samples (19). The age-wise distribution showed that the majority of isolates (32%) were in the 21–30 years age group, which correlates with increased healthcare

exposure and higher incidence of UTIs and reproductive tract infections in this age group. Comparable age distribution patterns have been reported in previous studies (20).

In the present study, urine was the most common sample source (56%), followed by wound/pus samples (31%) and blood (11%). This finding is consistent with studies by Bora et al. (2018) and Raina et al. (2020), which also reported urine as the predominant source of CoNS isolates (21,22). The high proportion of urinary isolates further supports the role of CoNS, particularly Staphylococcus saprophyticus, as an important pathogen in UTIs. The presence of CoNS in blood samples emphasizes their role in bloodstream infections, especially in hospitalized and immunocompromised patients.

The antimicrobial susceptibility pattern observed in the present study revealed complete resistance to penicillin (100%), which is in line with global trends indicating widespread β-lactam resistance among CoNS isolates. Similar findings were reported in studies from the Philippines and India, where penicillin resistance ranged from 90–100% (23). High resistance was also observed for cotrimoxazole (59%) and ciprofloxacin (53%), indicating limited effectiveness of commonly used antibiotics. On the other hand, linezolid (92%) and doxycycline (79%) showed high sensitivity, suggesting their continued efficacy against CoNS infections. These findings are consistent with recent studies that reported excellent susceptibility of CoNS to linezolid and glycopeptides (24).

Methicillin resistance was observed in 72% of isolates in the present study, which is comparable to findings from studies conducted in Iran and India, where MRCoNS prevalence ranged from 70–76% (25). The high prevalence of methicillin resistance is a major concern, as MRCoNS strains are often associated with multidrug resistance, limiting treatment options. In contrast, some earlier studies have reported lower prevalence rates ranging from 30–60%, indicating geographical variation in resistance patterns (26). The increasing trend of MRCoNS highlights the need for continuous surveillance and stringent infection control measures.

The distribution of methicillin resistance across clinical samples showed higher resistance among urine (78.6%) and wound samples (67.7%), indicating that MRCoNS are widely distributed across different infection sites. However, the association between sample type and methicillin resistance was not statistically significant ($p>0.05$), suggesting that methicillin resistance is uniformly prevalent across different clinical specimens.

The antibiotic susceptibility pattern of MRCoNS isolates revealed high resistance to cotrimoxazole (63.9%) and ciprofloxacin (61.1%), similar to findings reported by Singh et al. (2022), where multidrug resistance was a common feature among MRCoNS isolates (27). Linezolid remained highly effective (90.3%), which is consistent with global studies emphasizing its role as a reserve drug for resistant Gram-positive infections (28). These findings underline the importance of rational antibiotic use to prevent further emergence of resistance.

Biofilm production was observed in 34% of isolates in the present study. This is relatively lower compared to studies by Shrestha et al. (2018), who reported biofilm production in 65.3% of isolates (29), and studies from hospital environments showing prevalence up to 50% (30). The variation in biofilm production may be due to differences in detection methods, sample sources, and geographical factors. Biofilm formation is a critical virulence factor that enhances bacterial survival and resistance, particularly in device-associated infections.

A significant association was observed between methicillin resistance and biofilm production ($p=0.009$), with an odds ratio of 4.28, indicating that MRCoNS isolates were more likely to produce biofilms. This finding is supported by studies demonstrating that biofilm-producing CoNS isolates exhibit higher rates of methicillin resistance due to the presence of resistance genes such as mecA (31). Biofilm formation provides a protective environment for bacteria, leading to reduced antibiotic penetration and increased resistance.

Furthermore, the association between biofilm production and clinical samples was also statistically significant ($p=0.001$), with higher biofilm production observed in urine and wound isolates. This finding highlights the role of biofilms in chronic and persistent infections, particularly those associated with medical devices and urinary tract infections.

The present study also identified inducible clindamycin resistance in a proportion of isolates, emphasizing the importance of performing D-tests before reporting clindamycin susceptibility. Similar observations have been reported by Yilmaz et al. (2007), who highlighted the clinical significance of detecting inducible resistance to avoid therapeutic failure (32).

Overall, the findings of the present study are consistent with global trends indicating increasing antimicrobial resistance and biofilm production among CoNS isolates. However, the relatively lower prevalence of biofilm production compared to some studies suggests possible regional variation and underscores the need for standardized detection methods.

CONCLUSION

The present study demonstrates that Coagulase Negative Staphylococci are significant pathogens associated with a wide range of clinical infections, particularly urinary tract and wound infections. A high prevalence of methicillin resistance and multidrug resistance was observed, with linezolid remaining the most effective antibiotic. Biofilm production, identified in a substantial proportion of isolates, showed a significant association with methicillin resistance, highlighting its role in enhancing bacterial virulence and persistence. These findings emphasize the need for routine antimicrobial susceptibility testing, detection of methicillin resistance and biofilm formation, and implementation of strict infection control measures to prevent the spread of resistant CoNS strains.

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