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ORIGINAL RESEARCH

Antibiotic Resistance and *mecA* Gene Characterization of Coagulase-negative *Staphylococci* Isolated from Clinical Samples in Nepal

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Lok Bahadur Shrestha
Narayan Raj Bhattarai
Keshav Rai
Basudha Khanal

Microbiology & Infectious Diseases, B. P. Koirala Institute of Health Sciences, Dharan, Sunsari 56700, Nepal

Background: Coagulase-negative *Staphylococci* (CoNS) are a significant cause of hospital-acquired and foreign-body-related infections. We conducted this research to assess methicillin susceptibility of CoNS by disc diffusion, agar dilution, and polymerase chain reaction (PCR) methods and to assess the antimicrobial susceptibility pattern.

Methods: We received 123 CoNS isolates from different specimens including blood, endotracheal tube, and central venous catheter. We performed sample processing, identification, and characterization following standard guidelines. Antimicrobial susceptibility was tested based on clinical and laboratory standards institute guidelines. We detected methicillin-resistant coagulase-negative staphylococci (MRCoNS) through *mecA* gene, disc diffusion method, and agar dilution method and compared the accuracy with PCR as reference.

Results: We detected eight species of CoNS with *Staphylococcus epidermidis* as the most common. Most of the samples were received from the intensive care unit and blood was the dominant specimen followed by endotracheal-tube aspirate. Seventy-one percentage of isolates were methicillin-resistant by PCR method; disc diffusion and agar dilution method detected methicillin resistance with an accuracy of 96.7% and 98.3%, respectively. Antimicrobial susceptibility revealed an association between the different origins of samples, and also among the types of sample. Similarly, a comparison of the degree of resistance of antimicrobial agents between *mecA* gene positive and negative isolates showed significant differences. Vancomycin, linezolid, and teicoplanin are still effective for treating MRCoNS.

Conclusion: CoNS are a crucial cause of human infections especially in an intensive care unit setup where the use of devices is common. Disc diffusion and agar dilution are reliable for the detection of MRCoNS. The degree of antimicrobial resistance is much higher in organisms obtained from intensive care unit and foreign-body-related infections.

Keywords: foreign-body-related infections, methicillin-resistant CoNS, *mecA* gene

Background

Coagulase-negative *staphylococci* (CoNS) are opportunistic bacteria that have emerged as a vital cause of hospital-acquired infections accounting for 30%.[1,2] They frequently cause bloodstream and prostheses-related infections.[3] A major surge in incidence of methicillin-resistant coagulase-negative staphylococci (MRCoNS) has occurred over the years. Currently, more than 70% of the CoNS worldwide are MRCoNS.[1,3,4] The major mechanism of methicillin resistance is production of penicillin-binding protein (PBP 2a) encoded by the *mecA* gene.[5] There are also some reports of *mecC* gene encoded methicillin resistance in

Correspondence: Lok Bahadur Shrestha
Microbiology & Infectious Diseases, B. P. Koirala Institute of Health Sciences, Dharan 56700, Sunsari, Nepal
Email drlok86@gmail.com

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Staphylococcus aureus and CoNS.[6,7] The options for treatment of these infections are limited. Although novel antibiotics like telavancin, dalbavancin, oritavancin and linezolid have been found effective, vancomycin remains the gold standard drug.[8] Due to the emergence of vancomycin-resistant *staphylococci*, the recommendation is to minimize use of this drug.[2,9] Therefore, it is mandatory for laboratories to segregate methicillin-susceptible and resistant strains to mitigate inappropriate use of vancomycin.[1,2,10]

Although cefoxitin disk diffusion and agar dilution test are useful screening methods, detection of *mecA* gene by molecular method remains a reference method of detecting methicillin resistance among CoNS.[2,11]

This study aims to assess the methicillin susceptibilities of CoNS by correlating the outcome obtained by the disk diffusion and agar dilution methods with *mecA* gene detection.

Methods

We carried out this study at B. P. Koirala Insitute of Health Sciences, Dharan, Nepal from January 2018 to December 2018. We received 123 CoNS isolates from diverse samples including blood (n=43), urine (n=30), endotracheal tube (n=24), pus (n=14), and central venous catheter (n=12). We confirmed the isolate as CoNS based on their colony characteristics, gram staining, slide coagulase, and tube coagulase. We used the Kloos and Bannerman method to further characterize the isolates up to species level phenotypically. We employed a battery of biochemical tests like urease, acetoin production, sugars like mannitol, maltose, sucrose, xylose, and trehalose and discs like novobiocin and polymyxin B for characterization.[12,13] All tests were performed twice following good clinical and laboratory practice guidelines.

Antimicrobial Susceptibility Testing[14,15]

We performed antimicrobial susceptibility test of the isolates by the Kirby–Bauer method adhering to clinical and laboratory standard institute (CLSI) guidelines against following antimicrobial discs: amikacin (10 µg), cefalexin (30 µg), ceftriaxone (30 µg), ofloxacin (5 µg), vancomycin (30 µg), linezolid (30 µg), and teicoplanin (30 µg) (HiMedia, India). We checked the quality of all the discs by testing them against *Staphylococcus aureus* ATCC 25923 before use.

Detection of *mecA* Gene by PCR[16–19]

For DNA extraction, we subcultured the isolates onto Mueller–Hinton agar. After growth, we suspended five colonies in 100 µL of Tris-EDTA buffer and heated at 100°C. We centrifuged the solution at 9000 xg for 30 seconds and used 2 µL of supernatant as template in a 50 µL reaction. We used primers (*mecA-F*: 5'-GTAGAA ATGACTGAACGTCCGATGA and *mecA-R*: 5'- CCAAT TCCACATTGTTTCGGTCTAA) based on methodology as described by Jaffe R et al.[17] The master mix consist of reaction buffer, MgCl2, dNTPs, *mecA* primers, Taq polymerase and distilled water. We amplified DNA in a thermocycler (Eppendorf, Germany) and electrophoresed amplicons on a 1.5% agarose gel with 0.5 µg/mL ethidium bromide. We visualized gel under ultraviolet illumination. The positive tests showed PCR product of 310 bp. We used CoNS ATCC 25923 as negative control and CoNS ATCC 43300 as positive control.

Detection of MRCoNS by Disc Diffusion

We used cefoxitin (30 µg) disc along with other antimicrobial discs in the Mueller–Hinton agar plate to detect methicillin resistance.[15]

Detection of MRCoNS by Agar Dilution Method

We detected MRCoNS by estimating minimum inhibitory concentration (MIC) of oxacillin against the isolates as described by CLSI guidelines.[20] We interpreted the results based on the breakpoints provided by the guideline.

Results

We obtained 123 CoNS isolates belonging to eight different species, including *Staphylococcus epidermidis* (n=51, 42%), *S. saprophyticus* (n=31, 25%), *S. haemolyticus* (n=11, 9%), *S. lugdunensis* (n=9, 7%), *S. capitis* (n=4, 3%), *S. hominis* (n=7, 6%) and *S. warneri* (n=5, 4%) and *S. schleiferi* (n=5, 4%). We collected majority of samples from intensive care units (n=58, 47%) followed by wards (n=46, 39%) [Table 1]. Regarding specimen type, we received most of the isolates from blood samples (n=43, 34%), followed by urine (n=30, 24%) and endotracheal tube aspirate (n=24,19.5%).

We detected *mecA* gene in 70.7% (n=87) of the isolates. We observed that disc diffusion and agar dilution method detected methicillin resistance with 96.7% and 98.3% accuracy (Table 2).

Table 1 Number of Organisms with Respect to the Origin of Sample

	Total Number	ICU (n=58)	Wards (n=46)	OPD (n=11)	Emergency (n=8)
S. epidermidis	51	26	19	3	3
S. saprophyticus	31	10	16	3	2
S. haemolyticus	11	6	3	1	1
S. lugdunensis	9	5	2	1	1
S. capitis	4	3	1	0	0
S. hominis	7	5	1	1	0
S. warneri	5	2	2	1	0
S. schleiferi	5	1	2	1	1

Abbreviations: ICU, intensive care unit; OPD, outpatient department.

Table 2 Comparison of Disc Diffusion and Agar Dilution Method with *mecA* Gene

		mecA Positive		Sensitivity	Specificity	PPV	NPV	Accuracy
		Positive	Negative					
Disc diffusion	Positive Negative	83 4	0 36	95.4%	100%	100%	90%	96.7%
Agar dilution	Positive Negative	85 2	0 36	97.7%	100%	100%	94.7%	98.3%

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

We compared the *mecA* gene positivity with respect to origin of sample which was statistically significant (*P*-value 0.0001). The antimicrobial susceptibility was also compared between them, which turned out to be significant (Table 3).

Similarly, we also compared *mecA* gene output with the type of specimen. There was a significant difference (*P*-value <0.0001) between the samples with respect to *mecA* gene positivity (Table 4).

Finally, we observed the degree of antimicrobial resistance among *mecA* gene positive and negative isolates. We found a highly significant differences between the comparison groups (Table 5).

Discussion

We obtained 123 clinically significant CoNS isolates, which were mostly from intensive care units and specimen was blood and device related (endotracheal tube and central venous catheter). Similar types of findings have been reported by many studies done in the past.[21–23] CoNS are one of the most prevalent organisms affiliated with healthcare-associated and device-associated infections.[24] Increased use of medical devices in the ICU makes the patient vulnerable to colonization with CoNS.[4,23]

We isolated eight different species of CoNS, with *S. epidermidis*, as the most common. Several studies have identified *S. epidermidis* as the most commonly isolated CoNS.[22,25–29] In contrast, some studies have suggested *S. capitis*[30] and *S. hemolyticus*[10,31,32] as the most common CoNS. *S. epidermidis* is the most common commensal of our skin and mucosa, and contamination through the devices during medical procedures are very common.[4] In this study, S. saprophyticus was the second-most prevalent organism, which was found mostly in urine specimens. According to literature, *S. saprophyticus* is a frequently isolated CoNS and a common cause of urinary tract infection.[4,21,31,33]

In this study, we carried out detection of methicillin resistance by PCR method and the *mecA* gene was encountered in 70.7% of the isolates. The finding is similar to Secchi et al,[34] Ferreira et al,[11] and Hussain et al.[35] Higher rate (87%) of MRCoNS has been reported by Hira et al.[36] However, some studies reported a low occurrence of *mecA* gene positivity.[37–39] The high prevalence of *mecA* gene positive isolates in our study might be due to the fact that most samples were received from intensive care units and almost all patients admitted in our hospitals are treated in primary care hospitals with several courses of antibiotics and referred here.

The prevalence of *mecA* gene positivity with respect to the origin of samples showed that 93% of patients from ICU were positive, while only 63% of patients from the ward and

Table 3 Antimicrobial Resistance of Isolates with Respect to Origin

Origin of Sample	Number of CNS	*mecA* Gene (%)	Resistance Percentage in									
			Amikacin	Cephalexin	Cefoxitin	Ceftriaxone	Ciprofloxacin	Azithromycin	Cotrimoxazole	Vancomycin	Linezolid	Teicoplanin
ICU	58	93	65	96	96	96	75	77	84	0	0	0
Ward	46	63	40	77	73	73	66	60	75	0	0	0
OPD	11	9	0	72	45	45	54	18	45	0	0	0
Emergency	8	25	12	60	25	25	62	12	62	0	0	0
P-value		0.0001	0.0001	0.001	0.0001	0.0001	0.4	0.0001	0.03	NA	NA	NA

Abbreviations: ICU, intensive care unit; OPD, outpatient department.

Table 4 Antimicrobial Susceptibility Pattern of Isolates with Respect to Type of Sample

Type of Sample	Number of Isolates	*mecA* gene Positive (%)	Resistance Percentage in									
			Amikacin	Cephalexin	Cefoxitin	Ceftriaxone	Ciprofloxacin	Azithromycin	Cotrimoxazole	Vancomycin	Linezolid	Teicoplanin
Blood	43	72	51	83	76	76	74	62	79	0	0	0
Urine	30	43	20	70	60	60	53	36	56	0	0	0
ETT	24	100	62	91	91	91	83	66	83	0	0	0
CVC tip	12	100	75	100	100	100	58	83	83	0	0	0
Pus	14	64	42	92	85	85	78	85	92	0	0	0
P-value		0.0001	0.004	0.007	0.01	0.01	0.1	0.007	0.04	NA	NA	NA

Abbreviations: ETT, endotracheal tube; CVC, central venous catheter; NA, not applicable.

Table 5 Comparison of Antimicrobial Resistance Between *mecA* Gene Positive and Negative

	Resistance Percentage in								
	Amikacin	Cephalexin	Cefoxitin	Ceftriaxone	Ciprofloxacin	Azithromycin	Cotrimoxazole	Vancomycin	Linezolid	Teicoplanin
mecA positive	55	95	95	95	76	70	82	0	0	0
mecA negative	27	58	38	38	55	41	61	0	0	0
P-value	0.006	0.0001	0.0001	0.0001	0.02	0.003	0.01	NA	NA	NA

Abbreviation: NA, not applicable.

6% of OPD were *mecA* gene positive (*P*-value <0.0001). Similar data were reported by Ehsan et al,[40] Singh et al,[32] and Avgald-Ohman et al.[41] The higher occurrence of the *mecA* gene in ICU patients is due to long hospital stay, frequent invasive medical procedures, use of multiple antimicrobials, and chronic debilitated patients.[4,42]

Our study showed a sensitivity of 95.4% and 97.7% with disc diffusion and agar dilution methods, while specificity was 100% for both. Similar conclusions have been revealed by Secchi et al[34] and Bhatt et al.[37] Ferreira et al showed that although the sensitivity of both tests was high, specificity was lower (91% for disc diffusion and 73.5% for agar dilution).[11] Contrary to our finding, Graham et al[39] revealed that disc diffusion and dilution methods are inadequate to detect methicillin resistance. However, they have used oxacillin disc for disc diffusion and MIC was studied using E-test, which is different from our study. Other studies suggest that disc diffusion and agar dilution methods are reliable methods for the detection of methicillin resistance in CoNS.[43,44]

The comparison of antimicrobial susceptibility between *mecA* gene positive and negative isolates showed statistical difference. Amikacin resistance was exhibited in 55% of *mecA* positive isolates, while negative ones demonstrated 27% resistance (*P*-value 0.007) and a similar pattern was seen among other antimicrobials. The finding is similar to several studies.[11,32,45–47] Origin-wise analysis of resistance pattern also showed a highly notable variation ie *P*-value <0.05 in amikacin, cephalexin, ceftriaxone, cotrimoxazole, azithromycin. The finding is coherent with other studies.[32,42,48] Similarly, sample-wise analysis of resistance pattern reveals a significant difference in the degree of antimicrobial resistance between device-related samples and other samples (urine, pus). The finding is consistent with several other studies.[23,49] Multiple hospital admissions, frequent instrumentations and the capacity to establish multilayered biofilms on the surfaces makes these organisms resistant to most antimicrobials.[4,23,24]

Our study also demonstrated no resistance against vancomycin, linezolid, and teicoplanin; hence, these drugs remain the mainstay of treatment for CoNS isolates. The finding is in concordance with several other studies.[23,25,32,49] However, some studies suggest that there has been an increase in the number of cases with glycopeptide resistance.[4,42,50] Although *S. aureus* receives more attention due to its virulence and methicillin-resistance, nevertheless, CoNS also deserves attention

from clinicians due to its increasing significance and resistance.[51]

Conclusion

CoNS are crucial etiological agent of human infections especially in the ICU setup where the use of medical devices is common. Disc diffusion and agar dilution methods are simple and reliable methods for the detection of MRCoNS. The degree of antimicrobial resistance is much higher in isolates obtained from the intensive care unit and foreign-body-related infections. Resistance is much higher among *mecA* gene producer isolates as compared to negative ones.

Ethical Clearance

Obtained from Institutional Review Committee, BPKIHS (456/075/76-IRC).

Disclosure

The authors report no conflicts of interest in this work.

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