IJPSR (2025), Volume 16, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 July 2024; received in revised form, 23 August 2024; accepted, 25 October 2024; published 01 January 2025

A STUDY OF BIOFILM FORMATION AND METHICILLIN RESISTANCE IN COAGULASE NEGATIVE *STAPHYLOCOCCUS* IN VARIOUS CLINICAL SAMPLES

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Keywords:

Coagulase negative Staphylococcus, Biofilm, Methicillin resistance, Multi drug resistance, Linezolid

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ABSTRACT: Coagulase negative Staphylococcus, always regarded as nonpathogenic common commensal of skin and mucous membrane, are emerging as ubiquitous endemic nosocomial and oppurtunistic pathogens in recent times. They usually are considered as culture contaminants, thus left untreated which lead to their increased pathogenic potential. Methicillin resistance in Coagulase negative Staphylococcus is of significant concern as these strains are also multi drug resistant. The purpose of this study was to estimate the frequency of Methicillin resistance in CoNS in clinical samples and determine their biofilm producing ability and *in-vitro* antimicrobial susceptibility. A total of 2240 various clinical samples like urine, pus, blood, swabs received during one year period from January to December 2023 were processed in clinical laboratory, Department of Microbiology, Andhra Medical College, King George Hospital, Visakhapatnam. CoNS were isolated and identified by biochemical reactions and methicillin resistant strains identified by cefoxitin and oxacillin disc diffusion method and tested for biofilm production by Congo red agar and tissue culture plate method. Antimicrobial susceptibility done by conventional Kirby Bauer disk diffusion method.326 isolates (14.5%) were identified as CoNS, biofilm production was seen in 109 of 326 strains (33.4%), methicillin resistance in 46 of 326 (14.1%). The highest percentage of MRCoNS was observed in urine samples 124 of 326 (38%). All the isolates were sensitive to Linezolid. Emergence of MR in CoNS poses a major health concern due to injudicious use of antibiotics which lead to increased growth rate of antimicrobial resistance in CoNS.

INTRODUCTION: Non aureus Staphylococci are subtle and nonspecific common organisms isolated in clinical microbiology laboratories. These culture contaminants have emerged as true pathogens causing urinary tract, blood stream and localised infections due to their biofilm producing abilities [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.16(1).154-57

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(1).154-57

Methicillin resistance in Coagulase negative Staphylococci has been a recent challenge in cases left untreated. Methicilin resistance is mediated by mecA gene, SCCmec gene which encodes for penicillin binding protein 2A (PBP 2A) resulting in reduced affinity for antibiotics [2].

Methicillin resistant strains are of major concern as they generally are intrinsically resistant to many other antibiotic classes like aminoglycosides, quinolones and even glycopeptides, which may be due to transfer of plasmid resistance [3]. The present study was determined to estimate the prevalence of biofilm production, methicillin resistance and

analyze the *in-vitro* susceptibility patterns in coagulase negative Staphylococcus isolates from various clinical samples in clinical laboratory, Department of Microbiology, Andhra Medical College, King George Hospital, Visakhapatnam.

MATERIALS AND METHODS: A total of 326 coagulase negative Staphylococcus were isolated from 2240 samples during one year period of January 2023 to December 2023 in clinical laboratory, Department of Microbiology, Andhra Medical College, King George Hospital. Sample distribution during this period was urine (1138), blood (568), pus (319), swabs (215) respectively. Conventional methods were opted in the study. Staphylococcal isolates were identified by preliminary Grams staining, subcultured on nutrient agar, blood agar, mannitol salt agar and growth tested for biochemical reactions like slide and tube coagulase production, catalase reaction, urease production; mannitol, mannose, glucose fermentation; Novobiocin, Polymyxin B disks, methyl red, vogues proskauer reactions. Biofilm production was tested by congo red agar and tissue culture plate method. Methicillin resistance was tested by oxacillin and cefoxitin disk duffusion methods [4]. Standard reference strain of *Staphylococcus aureus* ATCC 25923 was used for quality control. Two consecutive blood cultures positive for coagulase negative Staphylococcus were only included in the study. Conventional kirby bauer disk diffusion method with 0.5 Mc Farland turbidity on 90mm petri plates was done to study the antimicrobial susceptibility pattern. Interpretation of the results was done according to CLSI and EUCAST guidelines [5].

RESULTS: A total of 326 coagulase negative Staphylococcus isolates were studied during one year period from January 2023 to December 2023 **Table 1**. The species identification was done on basis of biochemical reactions **Table 2**. Staphylococcus epidermidis (46.6%) was the commonest isolate, followed by Staphylococcus saprophyticus (20.9%) **Table 3**. Among 326 isolates, 109 (33.4%) were biofilm producers; out of which, 93 (85.3%) were confirmed by Congo Red Agar method and 109 (100%) by Tissue Culture Plate method. 217 (66.6%) were biofilm non forming. Biofilms were seen produced mostly in Staphylococcus epidermidis, Staphylococcus lugdunensis. Cefoxitin resistance in coagulase negative Staphylococcus was observed in 152 isolates (46.6%). Higher resistance was seen with PenicillinG (98.8%), Amoxyclav (96.3%), Nitrofurantoin (65.6%), Fosfomycin (55.2%), Cotrimoxazole (51.5%). Lowest resistance was recorded in Vancomycin (0.6%).

TABLE 1: DISTRIBUTION OF COAGULASE NEGATIVE STAPHYLOCOCCUS IN CLINICAL SAMPLES, n=326

Sample type	Total	Percentage %
Urine	124	38
Blood	102	31.3
Pus	54	16.6
Swabs	46	14.1

TABLE 2: BIOCHEMICAL REACTIONS OF COAGULASE NEGATIVE STAPHYLOCOCCAL ISOLATES

Test	*S. epidermidis*	*S. lugdunensis*	*S. saprophyticus*	*S. hominis*	*S. hemolyticus*
Hemolysis	-	+	-	-	-
Nitrate reduction	+	+	-	+	+
Urease	+	+	+	+	+
Tube coagulase	-	+	-	-	-
Novobiocin	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
Mannose	+	+	-	+	+
Mannitol	-	-			
Polymyxin B	Resistant	Sensitive	Sensitive	Sensitive	Sensitive
Methyl Red	+	-	-	-	-
Voges Proskauer	+	-	-	-	-
Glucose	+	+	+	+	+
PYR	-	-	-	-	+

TABLE 3: DISTRIBUTION OF TOTAL COAGULASE NEGATIVE STAPHYLOCOCCAL ISOLATES, n=326

Name of the isolate	Total isolated	Percentage %
Staphylococcus epidermidis	152	46.6
Staphylococcus lugdunensis	55	16.9
Staphylococcus saprophyticus	68	20.9

Staphylococcus hominis	18	5.5
Staphylococcus hemolyticus	33	10.1

DISCUSSION: A total of 326 clinically significant isolates of coagulase negative staphylococci were included in the study. 5 species of non aureus isolates were identified like *Staphylococcus epidermidis* (46.6%), *Staphylococcus saprophyticus* (20.9%), *Staphylococcus lugdunensis* (16.9%), *Staphylococcus hemolyticus* (10.1%), *Staphylococcus hominis* (5.5%). *Staphylococcus epidermidis* was isolated from various clinical samples like urine, blood, pus [6] and *Staphylococcus saprophyticus* mostly from urine samples; which may be attributed to its colonization of rectum and urogenital tract [7].

In our study, 109 isolates were biofilm producers detected by qualitative congo red agar method and quantitative tissue culture plate method. 100% biofilm production was detected by tissue culture plate method, which implies it as sensitive and specific method in this study. Most common biofilm producers were *Staphylococcus epidermidis* (72/109, 66.1%), *Staphylococcus lugdunensis* (32/109, 29.3%). Biofilm production in Gram positive bacteria involves a complex process with several factor contributions like, quorum sensing, protein A and teichoic acids, bacterial adhesins, colonization producing extracellular polymeric substances [8]. These provide protection from antibiotics and host immune responses [9, 10]. Antimicrobial susceptibility pattern in this study was done by *in-vitro* conventional kirby bauer disc diffusion method on overnight incubation at 37°C **Table 4**. Highest sensitivity to linezolid (100%). Methicillin resistance was studied in accordance with cefoxitin 30µg disk diffusion assay which is used as a surrogate marker for the determination of mecA gene. Isolates with cefoxitin zone of inhibition < 23mm were considered methicillin resistant, which is 46.6% in this study. Multidrug resistance in coagulase negative Staphylococcus is also carried out on a Staphylococcal Cassette Chromosome (SCC) which almost always includes the mecA gene for resistance to semi synthetic penicillins (SCCmec) [11].

Vancomycin susceptibility was confirmed by MIC against the isolates [12]. Vancomycin resistance in this study was documented in Staphylococcus epidermidis isolates [13]. Linezolid was the drug of choice in this study, it is a bactericidal agent which acts by inhibiting bacterial protein synthesis interfering the translational phase. Linezolid attaches to the 23S bacterial ribosomal RNA of the 50S subunit, preventing formation of a functional 70S initiation complex [14]. Most of the methicillin resistant coagulase negative staphylococcal isolates exhibit multi drug resistance [15, 16] which might be due to overlook of strains as contaminants thereby delaying treatment or indiscriminate and irrational antibiotic therapy [17].

TABLE 4: ANTIMICROBIAL SUSCEPTIBILITY OF TOTAL ISOLATES, N=326

Antimicrobial agent	Total sensitive	Total resistant	Resistance percentage %
Penicillin G	04	322	98.8
Amoxyclav	12	314	96.3
Cefoxitin	174	152	46.6
Amikacin	164	162	49.7
Ceftriaxone	204	122	37.4
Ciprofloxacin	228	98	30.1
Azithromycin	304	22	6.7
Cotrimoxazole	158	168	51.5
Vancomycin	324	02	0.6
Linezolid	326	0	0
Clindamycin	228	98	30.1
Teicoplanin	319	07	2.1
Nitrofurantoin	112	214	65.6
Fosfomycin	146	180	55.2

CONCLUSION: The early identification of methicillin resistance in coagulase negative staphylococci is essential to combat multidrug resistance. Tissue culture plate method is sensitive method for biofilm detection and Linezolid is the drug of choice in this study. Injudicious use of

higher antibiotics like vancomycin should be restricted for treatment of minor infective cases. Incidence of methicillin resistant non aureus strains is an alarming factor which should not be neglected in clinical scenarios.

ACKNOWLEDGEMENT: The author would like to thank the contribution of laboratory personnel during the study period.

Funding: The research did not receive any specific funding from any source.

CONFLICTS OF INTEREST: The author declares that no conflicts of interest exists.

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How to cite this article:
Vani TMSSS and Sujatha N: A study of biofilm formation and methicillin resistance in coagulase negative Staphylococcus in various clinical samples. Int J Pharm Sci & Res 2025; 16(1): 154-57. doi: 10.13040/IJPSR.0975-8232.16(1).154-57.