Open Access

Journal of Clinical Immunology and Microbiology

Research Article

Antibiotic Profile of Biofilm Producing Coagulase Negative *Staphylococci* Isolated from Various Clinical Specimens

Anamika Vyas[1], Etisha Nawani[2], Sheethal S[3*]

[1]Professor and Head of Department, Geetanjali Medical College and Hospital, Udaipur, India

[2]Assistant Professor, FH Medical College, Tundla, India

[3]Assistant Professor, Geetanjali Medical College and Hospital, Udaipur, India

[*]**Corresponding Author:** Sheethal S, Assistant Professor, Department of Microbiology, Geetanjali Medical College and Hospital, Hiran Magri Extension, Manwakhera, NH-8 Bypass, Near Eklingpura Chouraha, Udaipur-313001, Rajasthan, India; Email: dr.sheethal_s@yahoo.com

Received Date: 03-04-2022; **Accepted Date:** 21-04-2022; **Published Date:** 28-04-2022

Abstract

Introduction: CoNS which are normal commensal of skin and mucous membranes of human, when presented with an opportunity, are known to cause infections. The most common species among the group are the *S. epidermidis*. Production of extracellular slime called as biofilm help these pathogens to adhere to surfaces and protect themselves from the actions of host immune cells and chemotherapeutic agents.

Objective: To identify the biofilm producing CoNS from various clinical specimens, their speciation and antibiogram.

Materials and methods: 100 strains of CoNS isolated from various clinical samples were subjected to species identification by ornithine decarboxylase test, nitrate reduction test, Voges-Proskauer test, urease test, sugar fermentation tests and susceptibility to novobiocin and polymyxin B drugs. Biofilm production was detected using congo red agar and antibiotic susceptibility was tested by disc diffusion interpreted according to CLSI.

Results: Out of a 100 CoNS, we speciated *S. epidermidis* (32%), *S. hemolyticus* (25%), *S. hominis* (14%), *S. capitis*, *S. lugdunensis* (11% each) and *S. cohnii* (7%). Biofilm producers

Vyas A | Volume 3; Issue 1 (2022) | JCIM-3(1)-044 | Research Article

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

were 48 in number with *S. epidermidis* the leading pathogen. Antibiotic susceptibility showed a multidrug resistance pattern with commonly used antibiotics such as erythromycin, ciprofloxacin and gentamicin. However, 100% sensitivities were noted for rifampicin, vancomycin and linezolid.

Conclusion: Times of assuming CoNS as a non-pathogen is now in the past. Speciation of CoNS and identifying their biofilm producing abilities need to be performed alongside their antimicrobial susceptibility profiles.

Keywords

Coagulase Negative Staphylococci; Biofilm; *S. Epidermidis*; Slime Production

Abbreviations

CoNS: Coagulase Negative Staphylococci; CRA: Congo Red Agar

Introduction

The Coagulase Negative Staphylococci (CoNS) are normal commensal bacteria of the skin, anterior nares, ear canals, respiratory and gastrointestinal mucous membranes of not only humans, but also animals [1]. Colonization of this bacteria on humans has been observed to occur as early as at birth with many strains inhabiting the skin and mucous membranes until death [2]. However, when the opportunity presents itself such as presence of intravascular and prosthetic devices, long-term hospitalization, critically ill patients or immunocompromised/ immunosuppressed hosts, CoNS act as pathogens causing nosocomial infections [3-5]. A direct combination of these factors have put CoNS on the 3rd position in the list of etiological agents of nosocomial blood stream infections [4].

Out of all the species of CoNS, *Staphylococcus epidermidis* is the most frequently isolated species singly accounting for more than 90% of the aerobic flora and has gained popularity in the past two decades as a very frequent cause of hospital-acquired infections [6,7]. A two part postulated pathogenic potential of CoNS has been attributed to (a) their ability to affix themselves on to any material made up of synthetic polymers and (b) production of an extracellular "slime" material [8]. Multicellular communities of bacteria immobilized and held by an extracellular polymeric matrix produced by the bacteria themselves are called biofilms [2,9]. Such matrices help the producing microorganisms against extraneous antibiotics used to treat infections and in hiding from the host immune reactions [10-12].

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

The disastrous effects of biofilm producing CoNS include reduced susceptibility of these microbes to some commonly used hospital disinfectants and most importantly lead to contamination of hands of medical personnel culminating in contaminated hospital equipment and surfaces frankly increasing the risk of dissemination and transmission of such strains in to the hospital environment [13-15]. Early identification and antibiotic susceptibility profiling of these opportunistic CoNS will help in implementing adequate hospital infection prevention and control models. Therefore, the present study was undertaken to compile the data regarding biofilm producing CoNS from various clinical samples and discern their antibiotic susceptibility profiles.

Materials and Methods

One hundred coagulase negative *staphylococci* were isolated from various clinical samples obtained from OPD and IPD departments of Geetanjali Medical College and Hospital, Udaipur. Isolates were initially identified by Gram's stain, catalase test, coagulase reaction, bacitracin resistance and furazolidone resistance. Gram positive cocci measuring approximately 1µm in size arranged in grape-like clusters showing a positive catalase test, a negative coagulase test and resistance towards both bacitracin and furazolidone antibiotic discs were presumptively considered as species of CoNS.

Further speciation was performed using ornithine decarboxylase test, nitrate reduction test, Voges-Proskauer test, urease test, sugar fermentation tests and susceptibility to novobiocin and polymyxin B drugs.

Culture of these CoNS isolates on Congo Red Agar (CRA) plates as previously described by Freeman, et al., phenotypically characterized biofilm production in them [16]. Biofilm producers developed as black crusty colonies on CRA, whereas non-producers formed red colonies.

Antibiotic susceptibility testing was performed by disc diffusion method and interpreted according to CLSI guidelines 2014. Commercial antibiotic discs (Hi-Media Laboratories Pvt. Ltd, Mumbai) and Mueller-Hilton agar (Hi-Media Laboratories Pvt. Ltd, Mumbai) medium were used.

Results

Clinical samples such as blood, sputum, ET secretions, bronchial aspirates, throat swabs, pleural fluid, urine, pus, wound swabs and vaginal swabs were collected until we had a 100 CoNS isolated. Their speciation led to the identification of *S. epidermidis* (32%), *S. hemolyticus* (25%), *S. hominis* (14%), *S. capitis*, *S. lugdunensis* (11% each) and *S. cohnii* (7%). The male

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

population (67%) showed higher numbers of CoNS isolation than the female patients. The commonly affected age group by CoNS was between 41-60 years (30%), affliction in age groups 21-40 years and ≤20 years was similar (24% and 22% respectively) with ages 61-80 years and ≥80 years less commonly affected (19% and 5% respectively). Of the 100 isolates, 48% were detected to be biofilm producing strains. Species distribution of biofilm producing CoNS and their antibiogram are denoted in Table 1 and Table 2 respectively. Table 3 demonstrates the comparative antibiotic resistance patterns between biofilm and non-biofilm producing isolates of CoNS. Significance between slime producing and non-slime producing CoNS was determined and p value was calculated to execute the null hypothesis.

Species	Isolates	Biofilm Producers	Percentage
S. epidermidis	32	19	59%
S. hemolyticus	35	13	52%
S. lugdunensis	11	6	55%
S. hominis	14	4	29%
S. capitis	11	4	36%
S. cohnii	7	2	29%
Total	**100**	**48**	**48%**

Table 1: Species distribution of biofilm producing CoNS (n=100).

Antimicrobial Agent	No. of Sensitive Isolates	Sensitivity Percentage
Penicillin G	8	17%
Cefoxitin	18	38%
Ciprofloxacin	14	30%
Levofloxacin	32	67%
Gentamicin	37	78%
Erythromycin	12	25%
Clindamycin	22	46%

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

Cotrimoxazole	25	53%
Tetracycline	41	86%
Rifampicin	48	100%
Vancomycin	48	100%
Linezolid	48	100%

Table 2: Sensitivity pattern of biofilm producing CoNS (n=48).

Antimicrobial Agent	Resistance Percentage of Biofilm Producing Isolates (n=48)	Resistance Percentage of Non-Biofilm Producing Isolates (n=52)
Penicillin G	83%	46%
Cefoxitin	62%	55%
Ciprofloxacin	70%	44%
Levofloxacin	33%	30%
Gentamicin	22%	19%
Erythromycin	75%	73%
Clindamycin	54%	65%
Cotrimoxazole	47%	53%
Tetracycline	14%	3%
Rifampicin	0%	0%
Vancomycin	0%	0%
Linezolid	0%	0%

Table 3: Comparison of resistance patterns between biofilm and non-biofilm producing CoNS.

Discussion

Resident flora long considered harmless to ordinary individuals, CoNS tend to cause serious infections in compromised hosts, especially patients with prosthetic valves, intravascular

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

catheters, cerebrospinal fluid shunts or prosthetic joints. The advancement in the field of medicine has acutely resulted in an increased number of compromised hosts making CoNS one of the most common causes of nosocomial infections [17].

The formation of biofilm is a relatively common phenomenon among many microorganisms and is influenced by multiple environmental factors. The most common among them are oxygen, availability of iron ions and high osmotic pressure [18]. When the biofilm is viewed three-dimensionally, the extracellular matrix constitutes 85% of the structure comprising of polysaccharides, proteins, enzymes, DNA, bacterial glycolipids and water. The remainder 15% is made up by cellular aggregates of the microorganism [19]. In staphylococci, the main molecule responsible for intercellular adhesion is a Polysaccharide Intercellular Adhesin (PIA) also known as a Poly-N-acetylglucosamine (PNAG) [20]. It has been postulated that this is one mechanism of adherence and colonization of prosthetic devices by CoNS [21].

This study was undertaken to estimate the approximate numbers of CoNS producing biofilms in our institute so that this knowledge can be applied clinically to identify these opportunistic organisms as pathogens.

From a total of 100 CoNS derived from various clinical samples, we observed 48 isolates to be biofilm producing. Although these indeed are high numbers, higher percentages of up to 60% and even 87% have been observed by researchers Boynukara, et al., and Soumya KR, et al., directly linking biofilm production as a virulence factor in CoNS infections [22-24]. However, authors Shubra, et al., Makhija, et al., and Mohan, et al., have reported 39%, 43% and 44% of biofilm producing CoNS in their respective studies [25-27]. Christensen, et al., have reported that slime production is higher in pathogenic CoNS than in their non-pathogenic counterparts [28]. Due to the unavailability of modified microtiter plate assay, however, we could not perform this method of biofilm detection in our study.

Variations in biofilm producing CoNS species vary from place to place depending on the most prevalent CoNS in the particular environment. However, *S. epidermidis* is the most frequently isolated CoNS species producing biofilms as was also an observation of our study (Table 1) [15,27,29-31]. The other species isolated in the descending order were *S. hemolyticus, S. lugdunensis, S. hominis, S. capitis and S. cohnii. S. epidermidis* producing slime are associated with symptomatic infection [30]. Wojtyczka RD, et al., duly noted in their study an indication of a strong predominance of *S. epidermidis* isolates over other CoNS strains from clinical samples [15]. Another notable observation made by them was that CoNS strains isolated from the general hospital environment did not show such lenience. Therefore, the authors concluded that hospital environments colonized by biofilm-forming *S. epidermidis* strains can be held responsible for the increased risk of nosocomial infections in such environments.

Sardar SA, et al., identified an association between the ability of an isolate to produce slime and its propensity to cause disease in their study [30]. Another noted association is slime

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

production by CoNS and their pathogenicity. The association of biofilm production and antibiotic resistance which was debatable has been linked to impaired penetration of the drug across the biofilm making a few authors disregard the biochemical and genetic mechanisms of drug resistance in biofilm quorums [28,31-33]. The least sensitive antibiotics against biofilm producing CoNS in our study were penicillin G (17%) and erythromycin (25%); moderate sensitivities were observed for the fluroquinolone levofloxacin (67%), aminoglycoside gentamicin (78%) and tetracycline (86%); and no resistance was observed for the last line antibiotics rifampicin, vancomycin and linezolid.

With the emergence of resistance mechanisms, penicillin G and drugs such as ampicillin and amoxicillin have been rendered useless in treating CoNS. Ciprofloxacin when tested against CoNS by authors Ashok Kumar, et al., and Wojtyczka RD, et al., showed sensitivity percentages of 86.4% and 100% respectively, unlike our study where we found better sensitivities to levofloxacin [15,17]. Gentamicin showed considerable sensitivity at 78% when compared to authors Ashok Kumar, et al., who demonstrated only 48.4% sensitive strains [17]. Last line drugs which include rifampicin, vancomycin and linezolid so far have shown 100% sensitivities which can change in the coming years as foretold by Schwalbe in 1987 [34]. This also highlights the need to save these antibiotics lest we are left with none to combat CoNS in the future.

At the time of our study, we compared the antibiotic resistance patterns between slime producing and non-slime producing CoNS and found that there was no significant difference between the two groups ($p = 0.687$). This consistent observation with null hypothesis further strengthens the notion of impaired penetration of drug into the biofilm rather than a biochemical/ genetic component. However, individual drug resistance for penicillin G, quinolones, gentamicin, erythromycin and tetracycline were higher in slime producers than the non-slime producing CoNS.

Conclusion

CoNS are identified as pathogens from various clinical samples, many of which are biofilm producers resistant to routinely used antibiotics. Identifying such species of CoNS and charting their antibiotic susceptibity profile will help in their timely treatment.

Conflict of Interest

The authors declare they have no competing interest and not any conflict of interest.

Vyas A | Volume 3; Issue 1 (2022) | JCIM-3(1)-044 | Research Article

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

References

1. Piette A, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134:45-54.
2. Izano E, Amarante M, Kher W, Kaplan J. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. Appl Environ Microbiol. 2008;74:470-6.
3. Chu VH, Woods CW, Miro JM. Emergence of Coagulase-Negative *Staphylococci* as a cause of native valve endocarditis. Clin Infect Dis. 2008;46:232-42.
4. Golia S, Telsang DB, Kamath B AS, Tiwari D. Speciation of clinically significant coagulase negative staphylococci and their antibiotic resistant patterns in a tertiary care hospital. Int J Res Med Sci. 2015;3:1242-6.
5. Konemann EW, Allen SD, Janda WM, Schreckeneger P. The gram positive cocci *Staphylococci* and related organisms. Colour Atlas Diagn Microbiol. 6th Edition. 2006:623-71.
6. O'Gara J, Humphreys H. Staphylococcus epidermidis biofilms: Importance and implications. J Med Microbiol. 2001;582-7.
7. Cogen AL, Nizet V, Gallo RL. Skin microbiota: A source of disease or defence? Brit J Dermatol. 2008;158:442-55.
8. Greenwood WT, Cooper B, Hails J, Jones K, Kwaku F, Shaw S, et al. Line zolid versus teicoplanin in the treatment of Gram positive infections in the critically ill: A randomized; double-blind, multicentre study. J Antimicrob Chemother. 2004;53(2):345-52.
9. Otto M. Staphylococcus epidermidis-the "accidental" pathogen. Nat Rev Microbiol. 2009;7:555-67.
10. Costa FS, Miceli MH, Anaissie EJ. Mucosa or skin as source of coagulase-negative staphylococcal bacteraemia. Lancet Infect Dis. 2004;4:278-86.
11. Klingenberg C, Aarag E, Ronnestad A, Sollid JE, Abrahamsen MD, Kjeldsen G, et al. Coagulase-negative staphylococcal sepsis in neonates-Association between antibiotic resistance, biofilm formation and the host inflammatory response. Pediatr Infect Dis J. 2005;24:817-22.
12. Oliveira A, Cunha MLRS. Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci. BMC Res Notes. 2010.
13. Kampf G, Löffler H, Gastmeier P. Hand hygiene for the prevention of nosocomial infections. Dtsch Ärztebl Int. 2009;106:649-55.
14. Presterl E, Suchomel M, Eder M, Reichmann S, Lassnigg A, Graninger W, et al. Effects of alcohols, povidone-iodine and hydrogen peroxide on biofilms of *Staphylococcus epidermidis*. J Antimicrob Chemother. 2007;60:417-20.
15. Wojtyczka RD, Orlewska K, Kępa M, Idzik D, Dziedzic A, Mularz T, et al. Biofilm formation and antimicrobial susceptibility of Staphylococcus epidermidis strains from a hospital environment. Int J Environ Res Pub Health. 2014;11(5):4619-33.
16. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989;42:872-4.
17. Kumar A, Singla A, Bhullar AS, Neki NS. Slime production and antibiotic sensitivity pattern of coagulase negative staphylococci isolated from clinical specimens. Int J Curr Res Med Sci. 2018;4(10):1-9.
18. Otto M. Staphylococcal biofilms. Curr Top Microbiol Immunol. 2008;322:207-28.
19. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Sci. 1999;284:1318-22.
20. Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H, et al. The intercellular adhesin involved in biofilm accumulation *Staphylococcus epidermidis* linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. J Bacteriol. 1996;178:175-83.
21. Francois P, Vaudaux p, Foster TJ. Host-bacteria interactions in foreign body infections. IOnfect Control Hosp Epidemiol. 1996;17:514-20.

Vyas A | Volume 3; Issue 1 (2022) | JCIM-3(1)-044 | Research Article

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104

22. Boynukara B, Gulhan T, Gurturk K, Alisarli M, Ogun E. Evolution of slime production by coagulase-negative staphylococci and enterotoxigenic characteristics of Staphylococcus aureus strains isolated from various human clinical specimens. J Med Microbiol. 2007;56(10):1296-300.
23. Soumya KR, Philip S, Sugathan S, Mathew J, Radhakrishnan EK. Virulence factors associated with Coagulase Negative Staphylococci isolated from human infections. 3 Biotech. 2017;7(2):1-0.
24. Ramakrishna P, Syed A, Ashthami V. Biofilm: Comparison between the *Staphylococcus aureus* and coagulase negative *staphylococcus* species isolated from a rural medical college hospital in North Kerala, India. Int J Curr Microbiol App Sci. 2014;3(1):23-9.
25. Shubra DS. Species distribution and antibiotic sensitivity pattern of coagulase negative staphylococci isolates from various clinical specimen. 2005
26. Makhija SV, Jalgoankar SV, Kher M. Slime producing staphylococci from clinical specimens: a simple diagnostic test. Indian J Path Microbiol. 1995;38(2):159-61.
27. Mohan U, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of Coagulase negative staphylococci isolated from various clinical specimens. Ind J Med Microbiol. 2002;20(1):45-6.
28. Christensen GD, Simpson WA, Jounger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase negative staphylococci to plastic tissue culture plate: a quantitative model for the adherence the staphylococci to medical devices. J Clin Microbiol. 1986;22:996-1006.
29. Deighton MA, Franklin JC, Spicer WJ, Balkau B. Species identification, antibiotic sensitivity and slime production of coagulase-negative staphylococci isolated from clinical specimens. Epidemiol Infect. 1988;101(1):99-113.
30. SA Sardar, M Singh, S Basireddy, S Ali, V Kabra. COgagulase negative staphylococcus among clinical isolate in a tertiary care hospital. Int J Pharm Bio Sci. 2015;6(1):229-36.
31. Terki IK, Hassaine H, Oufrid S, Bellifa S, Mhamedi I, Lachachi M, et al. Detection of icaA and icaD genes and biofilmformation in Staphylococcus spp. isolated from urinary catheters at the University Hospital of Tlemcen (Algeria). African journal of microbiology research. 2013;7(47):5350-7.
32. Neishimura S, Tsurumoto T, Yonekura A, Adachi K, Shindo H. Antimicrobial susceptibility of staphylococcus aureus and Staphylococcus epidermidis biofilms isolated from infected total hip arthroplasty case. J Orthoped Sci. 2006;11:46-50.
33. O'Gara J, Humphreys H. Staphylococcus epidermidis biofilms: Importance and implications. J Med Microbiol. 2001;50:582-7.
34. Schwalbe RS, Stapleton JT, Gilligan PH. Emergence of vancomycin resistance in coagulase negative staphylococci. N Engl J Med. 1987;316:927-31.

Citation: Sheethal S, et al. Antibiotic Profile of Biofilm Producing Coagulase Negative Staphylococci Isolated from Various Clinical Specimens. J Clin Immunol Microbiol. 2022;3(1):1-9.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3104