ISSN: 0975-1556

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(1); 666-671

Original Research Article

Prospective Observational Research to Determine Antimicrobial Susceptibility and Biofilm Production among Coagulase Negative Staphylococci

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Received: 18-10-2021 / Revised: 05-12-2021 / Accepted: 12-01-2022
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Conflict of interest: Nil

Abstract

Aim: To determine antimicrobial susceptibility and biofilm production among coagulase negative staphylococci at a tertiary care hospital.

Materials and Methods: This prospective observational study was carried out in the Department of Microbiology, Patna Medical College, Patna, Bihar, India for 10 months. Purely isolated CoNS from various clinical samples from both outpatients and inpatients included in this study. All the test strains were subjected to antimicrobial susceptibility testing. The ability to produce biofilm was detected by tube adherence method.

Results: Most of the isolates were from pus swabs (40) followed by sputum samples (27). All the test strains were resistant to penicillin. Methicillin resistant was seen in 70% and mec A gene was present in 69% of the isolates. Majority of the strains were sensitive to ceftriaxone (77%), cefepime (69%), vancomycin (78%), cefaperazone – sulbactum (96%), piperacillin-tazobactum (99%). Only one CoNS strain isolated from blood sample showed resistant to piperacillin – tazobactum. Of the total 100 isolates of CoNS, 38% showed moderate biofilm formation by tube adherence method. 32% of isolates did not form biofilm. All the isolates from blood samples showed moderate (10/14) and strong (4/14) biofilm formation. Among non-biofilm producers 20% were MS CoNS isolates and 12% were MRCoNS. 60% of biofilm producers were MRCoNS and 8% were MSCoNS.

Conclusion: As Coagulase negative Staphylococci are exhibiting multi drug resistance and are able to form biofilm, these organisms causing a major challenge for the physicians. Hence, such problems can be prevented by detection of biofilm producers and appropriate antibiotic doses modification.

Keywords: Coagulase negative Staphylococci, biofilm, antibiotics.

Introduction

Coagulase negative staphylococci (CoNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections [1]. As the pathogenic significance increases, it becomes important to learn about the epidemiology and pathogenic potential of

individual species [2]. An important step in the development of catheter or implant-associated infections caused by CONS is the adhesion and attachment of these bacteria to biomaterial surfaces [3]. The capacity to adhere to polymer surfaces and consequent biofilm production are the main virulence factors of CoNS [4]. Biofilms are the microbial communities of the surface-attached cells, which are embedded in a self-produced extracellular polymeric matrix [5]. The microbial biofilms pose a serious health problem as the microorganisms in the biofilm are difficult to treat with antimicrobial agents. The decreased susceptibility to antimicrobial agents within biofilm arises from multiple factors such as decreased diffusion of antimicrobial agents, reduced bacterial growth rates and local alteration of microenvironment that may impair activity of antimicrobial agent [6]. Biofilm producing strains are found to be more resistant to almost all groups of antibiotics as compared to biofilm nonproducing strains [7].

Materials and Methods:

This prospective observational study was carried out in the Department of Microbiology, Patna Medical College, Patna, Bihar, India for 10 months

Methodology:

All purely isolated CoNS from various clinical samples from both outpatients and inpatients like urine, pus swabs, exudates, sputum, blood etc based on conventional methods. All the test strains were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method. Methicillin resistance was tested with Oxacillin and presence of mecA gene was tested cefoxitin antimicrobial susceptibility was read by following CLSI guidelines [8]. The ability to produce biofilm was detected by tube adherence method.

Tube adherence method:

The obtained bacterial pure isolates (loopful of bacteria) were inoculated into Trypticase soy broth supplemented with 1% glucose (TSBglu) and incubated for 24 hours at 37◦C. Tubes were decanted and washed with PBS (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed, and tubes were washed with deionized water and the experiment was done in triplicate manner. The controls for strong biofilm production and no biofilm production were S. epidermidis ATCC 35984 and S. epidermidis ATCC 12228 respectively.

Tubes were then dried in an inverted position and observed for biofilm formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Based on the intensity of the color formed, tubes were examined, and the amount of biofilm was scored as 0-absent, 1- weak, 2- moderate or 3-strong. All the tests were done as per standard operative procedures [9].

Results:

Most of the isolates were from pus swabs (40) followed by sputum samples (27) as shown in Table 1.

Table 1: Distribution of CoNS

Sample	Number (%)
Urine	19(19%)
Sputum	27(27%)
Blood	14(14%)
Pus swabs	40(40%)
Total	100(100%)

Table 2: Antimicrobial susceptibility of isolated CoNS

Antimicrobial	Pen		Ox		Cfx		Van		Ctr		CfS		PIT		Le		Cot		Cpm	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Urine	-	19	4	15	9	10	19	0	12	7	19	-	19	-	11	8	13	6	17	2
Sputum	-	27	10	17	7	20	19	8	18	9	27	-	27	-	21	6	22	5	26	1
Blood	-	14	1	13	6	8	10	4	8	6	12	2	13	1	10	4	4	10	5	9
Pus swabs	-	40	15	25	9	31	30	10	39	1	38	2	40	-	22	18	18	22	21	19
%age	0	100	30	70	31	69	78	22	77	23	96	4	99	1	64	36	57	43	69	131

Table 3: Categories of Biofilm formation by test strains

Urine	No biofilm 8	Weak 5	Moderate 4	Strong 2	Total 19
Sputum	16	5	4	2	27
Blood	-	-	10	4	14
Pus swabs	8	7	20	5	40
Total	32(32%)	17(17%)	38(38%)	13(13%)	100

Table 4: Comparison between biofilm producers and non-biofilm producers with methicillin susceptibility

Methicillin resistant (MR)	Non biofilm producers 12(12%)	Biofilm producers 60(60%)	Total 72
Methicillin sensitive (MS)	20(20%)	8(8%)	28
Total	32	68	100

All the test strains were resistant to penicillin. Methicillin resistant was seen in 70% and mec A gene was present in 69% of the isolates. Majority of the strains were sensitive to ceftriaxone (77%), cefepime (69%), vancomycin (78%), cefaperazone – sulbactum (96%), piperacillin- tazobactum (99%). Only one CoNS strain isolated from blood sample showed resistant to piperacillin – tazobactum as shown in Table 2.

Of the total 100 isolates of CoNS, 38% showed moderate biofilm formation by tube adherence method. 32% of isolates did not form biofilm. All the isolates from blood samples showed moderate (10/14) and strong (4/14) biofilm formation as shown in Table 3.

Among non-biofilm producers 20% were MS CoNS isolates and 12% were MRCoNS. 60% of biofilm producers were MRCoNS and 8% were MSCoNS as shown in Table 4.

Discussion:

The present study was done on 100 CoNS strains which were isolated purely from pus swabs (40%), sputum (27%), urine (19%) and blood (14%) samples. It was comparable with a study by Radhika et al [10]. (Pus- 41.35%). Though isolates of CoNS were more from pus samples (49/96) in a study by Tilakavarthy et al [11] the percentage (51.04%) was high when compared to our study. It was also observed in our study that more isolates were from inpatients than outpatients, which represents hospital stay and medical interventions can precipitate colonization of CoNS. CoNS gaining much importance in clinical settings as it frequently being reported from clinical specimens with multitude of drug resistance. Therapeutic options for the treatment of CoNS are limited because the vast majority of clinically recovered isolates are methicillin resistant [12]. In our present study all the isolates were penicillin resistant (100%). The same was observed in a study by

Hasanv and et al [13] but it was 96.1% in Sowmya et al [14] 99.3% of CoNS, isolated from hospital environment showed resistant to penicillin in a study by rathanin et al [15]. Methicillin resistant was 80.31% in present study, and it was comparatively less in studies by Sowmya et al [16] –(70%), Radhika et al [10]. (60.71%) and Shrestha et al [17]. (58%). vancomycin susceptibility in present study was less (84.97%) when compared to Radhika et al [10]. (100%), Hasanvand et al [13]. (100%), Shrestha et al [17]. (100%), and Sowmya et al [16]. (93%). Very less percentage of resistant was observed with piperacillin – tazobactum (1%) and ceftriaxone – sulbactum (4%) in present study, explained that resistant to beta lactam drugs could be overcome by administration drugs along with beta lactamase inhibitors. This could be helpful to the clinician in treating patients by choosing empirical antimicrobials correctly. Along with exhibiting multi drug resistance CoNS are known to have the ability to form polysaccharide intercellular adhesin and chemically diverse biofilm,[14] which is formed by a four-step process involving attachment, accumulation, maturation, and detachment, that's why today CONS represents one of the major pathogens among immune compromised and hospitalized individuals, with a considerable impact on morbidity and mortality [10] a also posing a major challenge for the physicians along with economic relevance as well [16]. Such problems can be prevented by detection of biofilm producers and appropriate antibiotic doses modification.

As test tube method is most suitable and reproducible method for detecting such strains [16]it was adopted in our present study. 76.68% of CoNS isolates showed ability to form biofilm in our present study. It was more in studies by Sowmya et al [14]. (87.5%), Rathanin et al [15]. (81.9%) and Bose et al [18] (45%), Fathima et al [19] (63.74%), Szcuska et al [20] (64%), Shrestha et al [14]. (65.38%), Bernard et al [21]. (45%) and (23.63%), Tilakavarthy et al [11]. (30.2%), Shareori et al [22] (36.3%). It was also observed in our present study that more isolates were in category of moderate biofilm producers (38%), whereas it was observed differently in a study by Fernando et al [23] that weak biofilm producers were more (34%) than moderate (10%) and strong (13%). The present study was also observed that all isolates from blood samples were moderate to strong biofilm producers. Hence pure isolation of CoNS from blood samples should be considered as pathogen rather than considering either contaminant or commensal. Many studies including our present study observed that antimicrobial resistance was high among biofilm producers than non-biofilm producers. In our present study it was observed that biofilm production was high in methicillin resistance (60%) than methicillin susceptible CoNS (8%) but it was 60.71% and 39.29% respectively in a study by Radhika et al [10]. The increased antibiotics resistance of biofilm producing strains might be due to their slow rate of metabolism and infrequent division resulting in decreased sensitivity to antibiotics targeted at cellular functions such as protein and DNA synthesis [17]. As there is a chance of persistent infections with organisms exhibiting biofilm production and multi drug resistance, these can land in untreatable conditions [19] and also creates a serious problem on public health [24]. The predominance of CoNS isolates in exhibiting (multi)resistance to antibiotics and antiseptics, as well as their capacity for biofilm production, is strongly indicative of selection processes facilitated by modern medicine, i.e., mainly from (over)use of antibiotics and insertion of foreign body devices [12].

Coagulase Negative Staphylococci are now being considered as emerging multidrug

resistant pathogens, hence, studies on their distribution, antibiotic sensitivity, and biofilm production are very important. The CoNS isolates of current study exhibited multiple antibiotic resistance similar to the other global reports. Studies on the prevalence of biofilm production and drug resistance can help to understand their role and interaction with each other and are necessary to identify new targets to develop therapeutic approaches. Further studies are needed to define the roles of the different components of undetermined biofilms and their regulation. Resistance to vancomycin can have serious impact because of the possibility to spread this to other bacterial strains. Thus, proper strategies should be adopted for the control and prevention of infections, and this requires close monitoring and periodic inspections of these potential multidrug resistant pathogens.

Conclusion:

As Coagulase negative Staphylococci are exhibiting multi drug resistance and are able to form biofilm, these organisms causing a major challenge for the physicians. Hence, such problems can be prevented by detection of biofilm producers and appropriate antibiotic doses modification. The issue of antibiotic resistance among CoNS needs to be addressed through a more rational use of existing antibiotics as well as the development of new antimicrobial agents.

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