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STANDARD TUBE (ST) METHOD INSTEAD OF TISSUE CULTURE PLATE (TCP) METHOD FOR DETECTION OF BIOFILM PRODUCTION AMONG COAGULASE NEGATIVE STAPHYLOCOCCI (CONS) ISOLATES IN A RURAL TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: The two main reasons of the increasing rate of CONS infections in recent years are the spreading of antibiotic resistance and increasing use of medical devices. Because of bioflim formation on medical devices, maximum of hospital acquired infections are caused by CONS. Biofilm formation also increases the resistance to antimicrobial agents and host defense mechanisms and because of that, it is very difficult to eradicate biofilm associated infections by conventional antibiotic treatment. Hence, the present study was undertaken with following aims and objectives. **Aims and Objectives:** To study biofilm production among the isolated species of CONS by Tissue culture plate (TCP) method and Standard tube method (ST) and to compare these methods for biofilm production. **Materials and Methods:** 180 CONS strains isolated from clinically significant samples were identified by different conventional methods and biofilm production was detected by TCP and ST method. **Results and Discussion:** Among 180 CONS isolates, predominant isolated species were S. epidermidis (40.56%), S.haemolyticus (25%). 60% and 54.44% CONS isolates were positive for biofilm production by TCP and ST method. Comparison of ST method with TCP method by Pearson chi square test showed strong association between TCP and ST and this distribution is statistically significant with P value of 0.006. **Conclusion:** ST method can be used in routine hospital laboratories for detection of biofilm production as it shows reliable results with good sensitivity and specificity.

KEY WORDS: Coagulase negative staphylococci (CONS), Tissue culture plate (TCP) method.

INTRODUCTION:

Coagulase negative Staphylococci (CONS) are ubiquitous in nature. Coagulase-negative Staphylococci are normal flora of human skin and mucous membranes, they have previously been considered nonpathogenic or contaminant having little clinical significance. The CONS maintain a benign relationship with their host and in certain situations like the natural barrier such as the skin is damaged via trauma, inoculation or implantation, they are considered pathogenic (Otto, 2004).

Over the last several decades, infection with CONS has been characterized as related to "medical progress" (Rupp, 1994) and because of the escalation in the rate of CONS infections in hospitals, medical interest in CONS has increased. But now they have been considered as significant potential pathogen responsible for hospital acquired infection. Accordingly, CONS, as human pathogens, are usually associated with healthcare settings and occur in patients who are immunocompromised or harboring indwelling polymer or metallic devices (Davenport, 1986).

The two main reasons of the increasing rate of CONS infections in recent years are the spreading of antibiotic resistance and increasing use of medical devices (Otto, 2004).Because of bioflim formation on medical devices, maximum of hospital acquired infections are caused by CONS. Biofilm formation also increases the resistance to antimicrobial agents and host defense mechanisms and because of that, it is very difficult to eradicate biofilm associated infections by conventional antibiotic treatment (Otto, 2004).

Hence, the present study was undertaken with the following aims and objectives.

AIMS AND OBJECTIVES:

1. To isolate species of CONS strains from clinically significant samples by conventional methods.

2. To study biofilm production among the isolated species of CONS by Tissue culture plate (TCP) method and Standard tube method (ST) and to compare these methods for biofilm production.

MATERIALS AND METHODS:

The present study was conducted in the department of Microbiology of Jawaharlal Nehru Medical College, which is a rural tertiary care hospital after taking approval of Institutional Ethic Committee. The study was a cross sectional study and the study was conducted from 29th September, 2016 to 28th September, 2018.

180 CONS strains were isolated from clinically significant samples (blood, urine, indwelling catheter, pus, body fluids) received in department of Microbiology and processed according to conventional methods.

CONS isolates from blood cultures should be correlated clinically and should be always interpreted with paired blood samples from two peripheral veins (Badampudi et al., 2016) and for other samples, repeated isolation of CONS in two consecutive samples is necessary (Puneet Bhatt, 2016).

CONS isolates from different clinically significant samples were initially identified by conventional methods such as colony morphology, gram staining, catalase and coagulase test (Colle et al., 2008)

Species identification of CONS was done by battery of biochemical tests such as ornithine decarboxylase test, sugar (mannitol, trehalose, mannose, xylose) fermentation test, phosphatase production, urease activity, nitrate reduction test, pyrrolidonyl arylamidase (PYR) test, acetoin production, novobiocin and polymyxin B (50 unit) sensitivity test etc, according to standard procedure (Washington et al., 2008).

Biofilm production was detected by phenotypic methods such as Tissue culture plate (TCP) method and Standard Tube (ST) method (Lucía E et al., 2003).

Biofilm production in test strains will be compared with reference strains (Lucía E et al., 2003).

- S.epidermidis ATCC 35983 (strong slime producer)
- S. hominis ATCC 35982 (Non slime producer)

RESULTS:

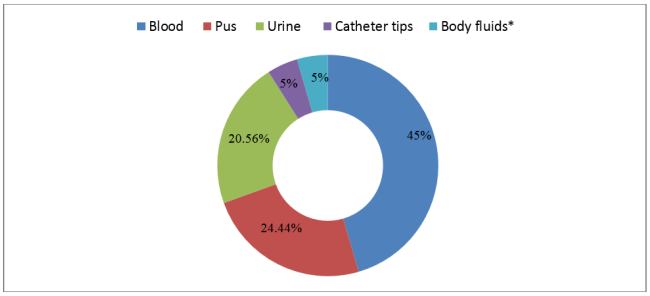

Figure 1: Sample wise distribution of CONS (n=180).

- **Body fluids include [CSF (n=1), Ascitic fluid (n=4), Pleural fluid (n=4)].**

Figure 1 shows sample wise distribution of CONS isolates from clinically significant samples. Out of 180 CONS isolates, 81(45%) isolates were from blood samples, 44(24.44%) isolates from pus samples, 37(20.56%) isolates from urine sam-

ples, 9 (5%) isolates from catheter tip samples and 9 (5%) isolates from body fluids respectively.

Table 1: Species distribution of CONS isolates (n=180).

Species	No of CONS isolates (n=180)
S.epidermidis	73(40.56%)
S.haemolyticus	45(25%)
S. schleiferi	20(11.11%)
S.lugdunensis	20(11.11%)
S.saprophyticus	11(6.11%)
S.xylosus	5(2.78%)
S.intermedius	3(1.67%)
S.warneri	2(1.11%)
S. hominis	1(0.56%)

Table 1 shows species distribution of CONS isolates. Out of 180 CONS isolates from different clinical samples, predominant isolated species were S. epidermidis 73(40.56%), S.haemolyticus 45(25%), S. schleiferi 20(11.11%) and S. lugdunensis 20(11.11%). Least commonly isolated CONS species were S.saprophyticus 11 (6.11%), S.xylosus 5(2.78%), S.intermedius 3(1.67%), S. warneri 2(1.11%) and S. hominis 1 (0.56%).

Biofilm production:

Biofilm production in CONS was detected by phenotypic methods such as Tissue culture plate (TCP) and Standard tube (ST) method.

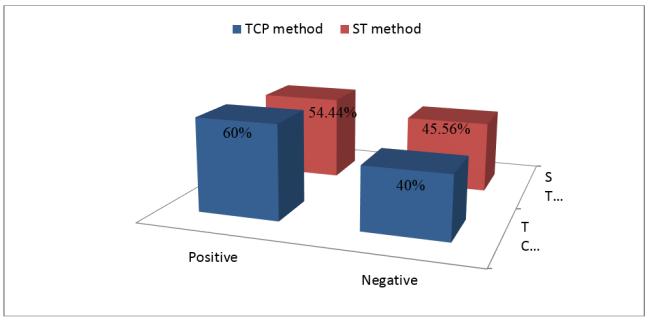

Figure 2: Biofilm production by phenotypic methods (n=180).

Figure 2 shows biofilm production by different phenotypic methods amongst 180 CONS isolates. Biofilm production was detected by TCP method and ST method. Out of 180 CONS isolates, 108 (60%) CONS isolates were positive and 72(40%) CONS isolates were negative for biofilm production by TCP method. 98 (54.44%) CONS isolates were positive and 82(45.56%) CONS isolates were negative for biofilm production by ST method.

So, from the table it was observed that TCP method showed higher biofilm production compared to the ST method.

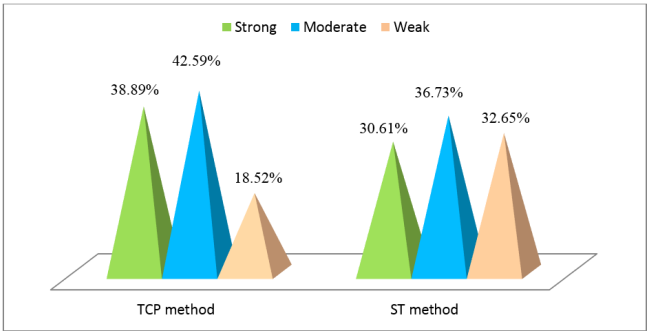

Figure 3: Grading of positive biofilm production by three different phenotypic methods.

Figure 3 shows grading of positive biofilm production by three different phenotypic methods. Amongst 108 biofilm producing CONS isolates by TCP method, 42(38.89%), 46(42.59%) and 20(18.52%) isolates showed strong, moderate and weak biofilm production respectively.

Amongst 98 biofilm producing CONS isolates by ST method, 30(30.61%), 36(36.73%) and 32(32.65%) isolates showed strong, moderate and weak biofilm production respectively.

Table 2: Species wise comparison of biofilm production by TCP and ST method.

Species	TCP method	ST method
S.epidermidis(n=73)	48(65.75%)	44(60.27%)
S.haemolyticus (n=45)	28(62.22%)	27(60%)
S. schleiferi (n=20)	11(55%)	10(50%)
S.lugdunensis (n=20)	11(55%)	9(45%)
S.saprophyticus(n=11)	6(54.54%)	6(54.54%)
S.xylosus(n=5)	2(40%)	1(20%)
S.intermedius(n=3)	2(66.67%)	1(33.33%)
S.warneri(n=2)	0(0%)	0
S. hominis(n=1)	0(0%)	0
Total(n=180)	108	98

Table 2 shows species wise biofilm production by TCP and ST method. Amongst 180 CONS isolates, 108 showed biofilm production by TCP method. Among these 108 CONS isolates, 48(65.75%) S. epidermidis followed by 28(62.22%) S. haemolyticus, 11(55%) S. schleiferi, 11(55%) S. lugdunensis, 6(54.54%) S. saprophyticus, 2(40%) S. xylosus and 2(66.67%) S. intermedius were biofilm producers by TCP method. Out of 180 CONS isolates, S. warneri (n=2) and S. hominis (n=1) were non biofilm producers by TCP method.

Amongst 180 CONS isolates, 98 were biofilm producers by ST method. Out of these 98 CONS isolates, 44(60.27%) S. epidermidis isolates followed by 27(60%) S. haemolyticus isolates, 10(50%) S. schleiferi isolates, 9(45%) S. lugdunensis isolates and 6(54.54%) S. saprophyticus isolates, 1(20%) S. xylosus and 1(33.33%) S. intermedius were biofilm producers by ST method. Out of 180 CONS isolates, S. warneri (n=2) and S. hominis (n=1) were non biofilm producers by ST method.

Out of 180 CONS isolates, 73 isolates were S. epidermidis. Amongst these 73 S. epidermidis isolates, 1 isolate was weak biofilm producer by ST method but non biofilm producer by TCP method.

So from the above table it was observed that biofilm production among CONS isolates by TCP and ST method was almost similar and there were no major differentiations among both of these methods.

DISCUSSION:

Once upon a time, CONS was considered as contaminant or non-pathogenic, but now-a-days, it has become one of the most important and commonest causes of opportunistic and hospital associated infections in severely ill and immunocompromised patients (Otto, 2004).

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In the present study, among 180 CONS isolates, 45% isolates were from blood samples, 24.44% isolates from pus samples, 20.56% isolates from urine samples, 5% isolates from catheter tip samples and 5% isolates from body fluids respectively. This is in accordance with the study done by Sadhvi Parashar et al. (Sadhvi Parashar, 2014) where 45.95% CONS isolates were from blood samples and 19.46% isolates were from urine samples. This is also in accordance with study done by Badampudi et al. (Badampudi et al., 2016) where 40% CONS isolates were from pus samples.

In the present study among 180 CONS isolates, from different clinical samples, predominant isolated species were S. epidermidis (40.56%), S.haemolyticus (25%), S. schleiferi (11.11%) and S. lugdunensis (11.11%). Least commonly isolated CONS species were S.saprophyticus (6.11%), S.xylosus (2.78%), S.intermedius (1.67%), S. warneri (1.11%) and S. hominis (0.56%). This is in accordance to study done by Badampudi et al. (Badampudi et al., 2016) where predominant isolated species were S. epidermidis (40%), S. haemolyticus (26%) and S. schleiferi (13%).

In present study among CONS isolates, biofilm production was detected by two different phenotypic methods such as TCP and ST method.

In present study, 60% CONS isolates were positive and 40 % CONS isolates were negative for production of biofilm by TCP method. This is in accordance with a study done by Devapriya F et al. (Devapriya F et al., 2014) where 64.4 % and 35.6% CONS isolates were positive and negative for biofilm production by TCP method.

Deka N et al. (Deka N et al., 2014) reported in their studies higher biofilm production by TCP method (83%) among CONS isolates.

In present study, 54.44% CONS isolates were positive for biofilm production by ST method. This is in accordance with a study done by Singh S et al. (Singh S et al., 2015) where 41.56% CONS isolates were positive for biofilm production by ST method.

In present study, 38.89% and 30.61% CONS isolates showed strong biofilm production by TCP method and ST method respectively. This is in accordance with a study done by Deka N et al. (Deka N et al., 2014) where 36% and 21% of CONS isolates showed strong biofilm production by TCP method and ST method respectively.

In present study, 42.59% and 36.73% of CONS showed moderate biofilm production by TCP method and ST method respectively. This is in accordance with study done by Deka N et al. (Deka N et al., 2014) where 47% and 36% of CONS isolates showed moderate biofilm production by TCP method and ST method respectively.

So from the above findings it was observed that TCP method showed higher biofilm production compared to the ST method.

TCP method is the gold standard method for biofilm production and when we compared ST method with TCP method for detection of biofilm production, ST method showed good kappa agreement and sensitivity and specificity of 89.81% and 98.61%. So, from the above findings, we can say that ST method can be compared to TCP method for biofilm production.

Compared to the TCP method, ST method is easy to perform, no need of any expensive instruments, cost effective, no need of any experts to read the results and can be done in routine hospital microbiological laboratories. On the other hand, TCP method is expensive, need of expensive instruments and microbiologists to read the results. That's why ST method can be readily available in routine laboratories and can be considered as the alternative method for biofilm detection instead of TCP method.

In the present study, detection of biofilm production by TCP and ST method showed almost similar results (TCP-60%, ST- 54.44%). But when we compared the grading of biofilm production by TCP and ST method, we found that 38.89% and 30.61% CONS isolates showed strong biofilm production by TCP method and ST method which was almost similar. The little bit differentiation of strong biofilm production between TCP and ST method was seen due to judgmental errors by different observers. This finding correlates with a study done by Thilakavathy et al. (Thilakavathy et al., 2015).

In present study, amongst all the CONS isolates, biofilm production by TCP method was seen in 65.75% S.epidermidis isolates followed by 62.22% S.haemolyticus isolates, 55% S.lugdunensis isolates and 54.54% S.saprophyticus isolates. This observation correlates with a study done by L.E. Alcarez et al. (L.E.Alcarez et al., 2003) where biofilm production by TCP method was seen in 57.14% S.epidermidis isolates followed by 37.5 % S.haemolyticus isolates and 83.83% S.saprophyticus isolates.

In present study, among all the CONS isolates biofilm production by ST method was seen in 60.27% S.epidermidis isolates followed by 60% S.haemolyticus isolates, 54.54% S.saprophyticus isolates and 45% S.lugdunensis isolates. This study correlates with a study done by L.E.Alcarez et al. (L.E.Alcarez et al., 2003) where 57.14% S.epidermidis isolates followed by 37.5 % S.haemolyticus isolates were biofilm producers by ST method.

Another study done by Badampudi et al. (Badampudi et al., 2016) reported among the CONS isolates, 55% S. epidermidis isolates were biofilm producers by ST method.

So from the above findings, it was observed that most predominant biofilm producing CONS isolates were S. epidermidis, S. haemolyticus, S. schleiferi, S.lugdunensis and S.saprophyticus and all the above mentioned CONS isolates showed almost similar biofilm production by both TCP and ST method.

CONCLUSION:

From the above study it can be inferred that ST method can be considered as a reliable, general screening method for biofilm detection instead of TCP, which is considered as the gold standard method as ST method showed good sensitivity and specificity, less expensive and can be routinely done in hospital laboratories followed by help to guide the physicians to pick proper antibiotic for patient treatment.

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