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A study of biofilm forming pathogens on endotracheal tube and their antimicrobial susceptibility patterns

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Abstract
This study aimed to detect the occurrence of biofilm forming isolates from endotracheal tube and to explore their antibiotic resistant pattern.

Introduction

Biofilm comprises of the crammed bacterial population by extra-cellular matrix (ECM) which possesses bacterial secreted polymers such as exopolysaccharides (EPS), extracellular DNA (e-DNA), proteins and amyloidogenic proteins[1,2]. EEM facilitates communication among the cells through biochemical signals such as acyl homoserine lactone in Gram-negative bacteria and oligopeptides in Gram-positive bacteria, in a phenomenon called as "Quorum sensing". Although biofilms play an important positive role in a variety of ecosystems, they also have many negative effects, including biofilm-related infections in medical settings. Biofilms are serious global health concern due to their abilities to tolerate antibiotics, host defence systems and other external stresses; therefore it contributes to persistent chronic infections[3,4]. It is estimated that about 65% of all bacterial infections are associated with bacterial biofilms[5]. These include both, device and non device associated infections. Data for device related infections have been estimated for several devices, such as: 2% for breast implants; 2% for joint prostheses; 4% for mechanical heart valves; 10% for ventricular shunts; 4% for pacemakers and defibrillator, and about 40% for ventricular-assisted devices[6]. Native valve endocarditis (NVE) is an inflammation caused by interaction of bacteria with the vascular endothelium and pulmonic valves of the heart. This is usually the result of *Streptococci*, *Staphylococci*, gram negative bacteria, and/or fungal infections[7]. In this condition microbial cells gain access to the heart and blood through the gastrointestinal tract, urinary tract and/or through the oropharynx. As the intact valve endothelium gets damaged by the microorganisms that attach to it, even after the bacteria have been cleared by the immune system a non bacterial thrombotic endocarditis (NBTE) develops at the injury location, as a result

a thrombus formation occurs, a condition where platelets, red blood cells and fibrin are aggregated[8]. Treatment of infections with biofilm producing bacteria is extremely difficult, requires higher doses or combination of antibiotics, and removal of foreign bodies when implicated in device related infections[9]. Early detection of biofilm producing organisms is therefore necessary to help manage ETT related infection which is one of the DRI (device related infection) and to reduce the spread of antimicrobial resistance. Thus, the present study was done to detect the occurrence of biofilm forming isolates from ETT and to explore their antibiotic resistance pattern.

Materials and Methods

This prospective observational study was conducted in the Department of Microbiology, Government Medical College and Hospital, Jammu (J&K). We collected Endotracheal tube tip from 30 patients admitted in ICU that were put on ETT for more than 2 days. Patients with ETT for less than 2 days were excluded from the study.

Sample Processing

ET tip received in bacteriological laboratory were inoculated in Blood agar and MacConkey agar by Roll plate method and incubated at 37˚C for 24 hours. The isolates were identified by colony morphology, gram staining and standard biochemical tests as per the departmental protocols.

Biofilm Detection

Biofilm detection was done by Congo Red Agar method where the plates of the congo red medium were inoculated with organisms to be tested and incubated with organisms to be tested and incubated aerobically for 24 hours at 37˚C. Congo Red Agar was composed of Brain heart infusion broth (37g/l), Sucrose (50g/l), Agar No.1 (10g/l), Congo Red (0.8g/l). Congo red stain was prepared as a concentrated solution and autoclaved seperately (121˚C for 15 minutes) from other medium constituents and then added when agar was cooled to 55˚C.

Procedure

Plates of the Congo Red Agar Medium (Hi Media Labs) were inoculated with organisms to be tested and incubated aerobically for 24 hours at 37˚C.

Interpretation

A positive result was indicated by black colonies with dry crystalline consistency [Figure 1]. Non slime producers usually remained pink, though occassional darkening at the centre of the colonies can be seen giving a "Bull eye appearance" [Figure 1].

Figure 1: Congo Red Agar Medium showing colonies of slime/non-slime producers

Tube Adherence Test

Two milliliter (ml) volumes of Brain Heart Infusion (BHI) broth supplemented with 1% glucose in 12×75mm borosilicate test tubes were inoculated with single colonies and incubated statically for 48 hours at 37˚C. The contents were decanted and washed with phosphate buffered saline (PBS), pH 7.2 and dried. One ml volume of 1% safranin solution was added. Each tube was then gently rotated to ensure uniform staining of any adherent material on the inner surface and the contents gently decanted to remove excess stain. The tubes were then washed with distilled water and placed upside down to drain [Figure 2]

Figure 2: Tube adherence test. Tube 1: Non adherent, Tube 2: Weakly adherent, Tube 3: Moderately adherent, Tube 4: Strongly adherent.

Result

Out of a total of 30 ET tip samples, 2 samples (6.6%) showed gram positive and 28 samples (93.3%) showed gram negative bacterias as the isolates [Figure 3]. Of the 2 gram positive samples, both the isolates were *Staphylococcus aureus spp.* Of the 28 gram negative samples, 6 isolates were *Klebsiella spp.*, 5 were *Acinetobacter spp.*, 5 were *Pseudomonas spp.*, 4 were *Citobacter spp.*, 4 were *Escherichia coli spp.* and 4 were isolated as *Proteus spp.*[Figure 4]

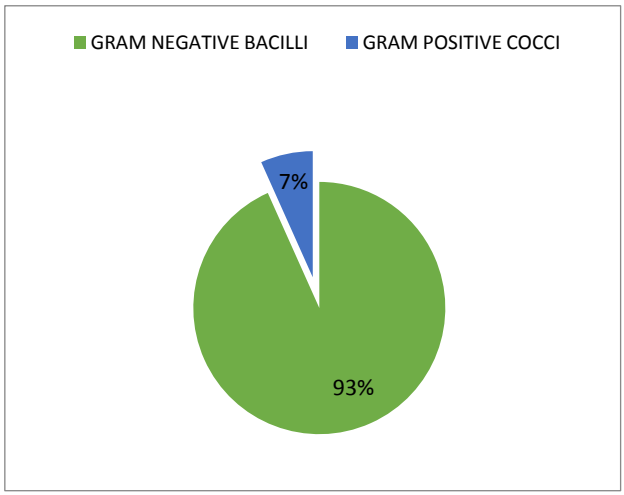


Figure 3: Percentage of Organisms Obtained

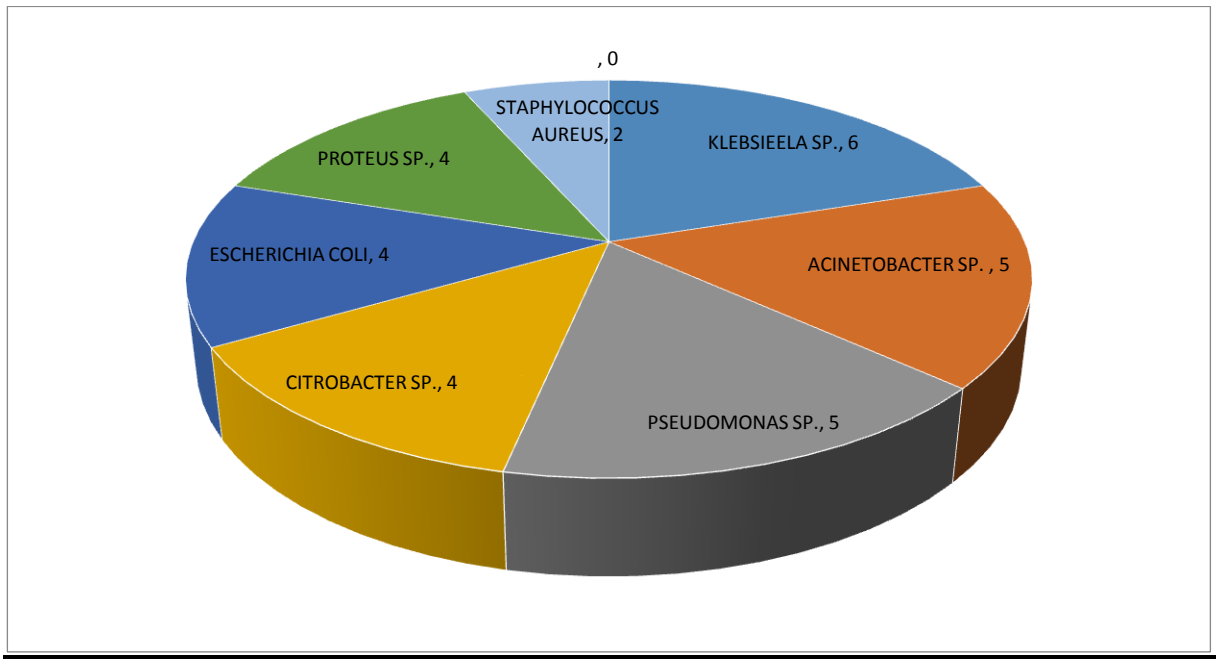


Figure 4: Type and Number of Organisms Isolated

Among the 2 different methods used, Congo red agar method isolated 25% of gram negative bacilli and 50% of gram positive cocci whereas Tube adherence method isolated 35.7% of gram negative bacilli and 50% of gram positive cocci [Figure 5 & 6]

Figure 5: Biofilm production by two different methods in Gram Negative Bacilli

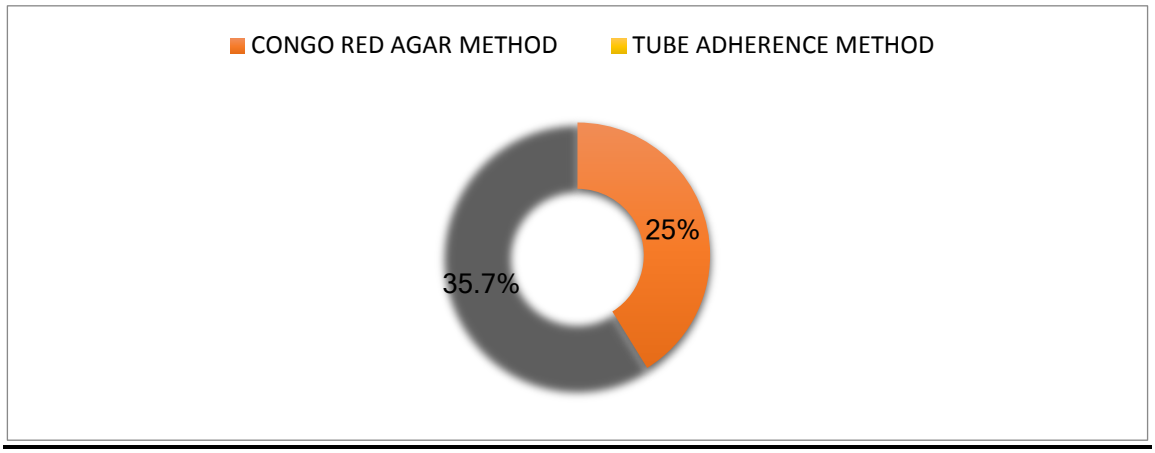
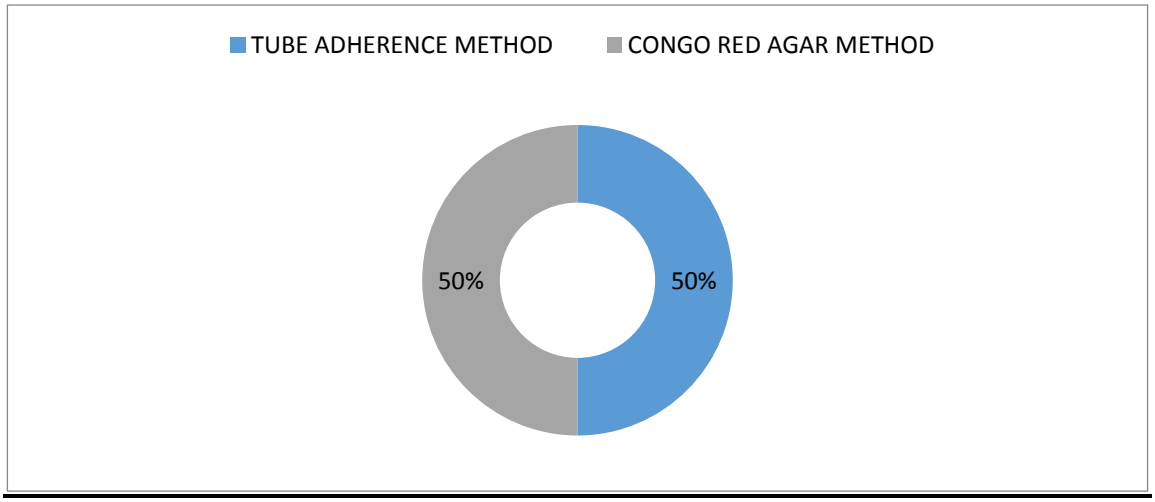
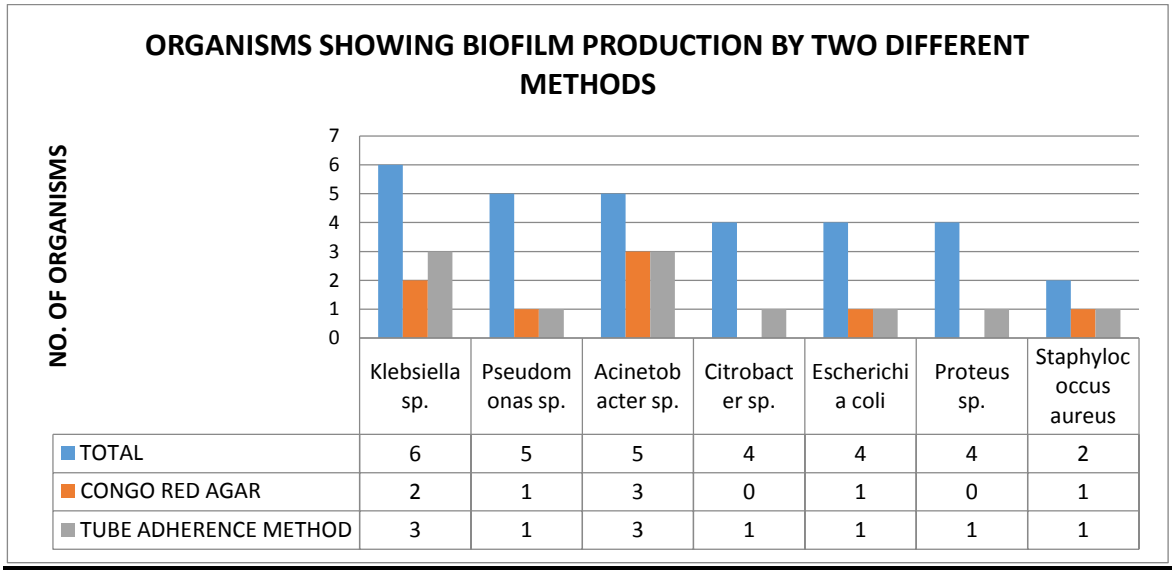


Figure 6: Biofilm production by two different methods in Gram Negative Bacilli



TA method detected 11(33.33%) biofilm producers and 19(63.7%) biofilm non-producers whereas CRA showed 8(26.7%) isolates as biofilm producers and 22 (73.3%) as non-producers [Figure 7].

Figure 7: Organisms showing biofilm production by two different methods



Antibiotic resistant pattern in gram negative and gram positive isolates has been shown in [Table 8] and [Table 9] respectively.

Table 9: Antibiotic resistant patterns in gram negative isolates (n=28)

ANTIBIOTICS	BIOFILM POSITIVE ISOLATES %	BIOFILM NEGATIVE ISOLATES %
AMPICILLIN	10O	1OO
AMOXYCLAVULINIC ACID	100	100
PIPERACILLIN TAZOBACTAM	70	11.11
CEFTRIAXONE	100	100
CEFEPIME	100	55.6
AZTREONAM	100	83.3
IMIPENEM	100	77.8
TOBRAMYCIN	10	5.6
NORFLOX	100	77.8
COTRIMOXAZOLE	80	16.7
CHLORAMPHENICOL	90	11.11
COLISTIN	0	0
POLYMIXIN B	10	0

Table 10: Antibiotic resistant patterns in gram positive isolates (n=2)

ANTIBIOTICS	BIOFILM POSITIVE ISOLATES %	BIOFILM NEGATIVE ISOLATES %
PENICILLIN G	100	100
CEFOXITIN	100	50
CIPROFLOXACIN	100	100
GENTAMYCIN	100	100
NORFLOXACIN	100	50
COTRIMOXAZOLE	100	100
VANCOMYCIN	0	0
CLINDAMYCIN	100	100
ERYTHROMYCIN	100	100
LINEZOLID	0	0
CHLORAMPHENICOL	100	0
TETRACYCLINE	100	0

Conclusion

1) Tube adherence method is more sensitive and a cost effective method as compared to CRA method.
2) Resistance is more common in case of biofilm producers than that of non biofilm producers.
3) Colistin and polymyxin B are the satisfactory drugs for gram negative bacilli whereas vancomycin and linezolid are effective in case of gram positive cocci.

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