Biofilm formation and multidrug resistance in nosocomial isolates of Acinetobacter

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Abstract
Purpose: To study the biofilm formation and to examine the correlation between antibiotic resistance and biofilm formation among the clinical isolates of Acinetobacter.
Materials and Methods: A total of 43 isolates of Acinetobacter collected from samples like peripheral venous catheter tips, urine from Foley's catheter, central venous catheter tips and endotracheal tube aspirates were preserved and processed. The tube method was performed to qualitatively detect Biofilm production. Antimicrobial susceptibility was done as per CLSI guidelines.
Place of Study and Study Period: Yenepoya medical college, Mangaluru and June 2015 to December 2015.
Study Design: Prospective
Results: Out of 43 isolates of Acinetobacter, 26(60%) showed biofilm production, among which 19 isolates (73%) were Multidrug resistant (MDR) and only 7 isolates (27%) were Non-MDR. Highest sensitivity was seen to colistin (100%) followed by imipenem (73.7%) and least sensitivity to ampicillin (0%) followed by ciprofloxacin (3.8%).
Conclusion: Antibiotic resistance was found to be significantly higher among biofilm producing Acinetobacter isolates than nonbiofilm isolates. Routine and advanced studies of the biofilm production will help in making better usage of the invasive devices with less critical complications.

Keywords: Biofilms, Acinetobacter, Multidrug resistance.

Introduction

Biofilm plays an important role in pathogenesis of device-related infections and drug resistance. Medical devices are being increasingly used in almost all fields of medicine for diagnostic and therapeutic procedures, moreso for managing critically ill patients.[1] However, the use of foreign material in the form of medical devices is almost invariably associated with a definitive risk of bacterial and fungal infections, that is, foreign body-related infections (FBRIs) for which one of the underlying mechanisms is biofilm formation.[2] The bacteria adhere to these surfaces and become sessile, secreting a slimy glue like substance for anchorage, forming biofilm which is a structured community of bacterial cells enclosed in a self-produced polymeric matrix adherent to the inert or living surfaces.[3]

The tendency of biofilm formation increases proportionately with the time for which the indwelling medical devices remain in place. 30% of biofilm forming bacteria are isolated from the indwelling medical devices such as endotracheal tubes, central venous catheters and urinary catheters.[4] Bacteria commonly isolated from these devices include *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, E.coli, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter* and *Pseudomonas aeruginosa.*[5] It has been observed that the susceptibility of a bacteria to the antimicrobial agents decreases with biofilm formation and also enhance the spread of antimicrobial resistance by facilitating a plasmid exchange due to the proximity of cells.[6]

Therefore, infection with multidrug resistant(MDR) or Pandrug resistant strains of bacteria like *Acinetobacter* and *Pseudomonas* are of great concern for hospitalized patients.[3] The contaminated reusable medical equipment such as humidifiers, respirometers, ventilator tubing, arterial pressure monitoring devices are important source of infection by *Acinetobacter.*

Acinetobacter has emerged as an important human nosocomial pathogen causing infections like ventilator-associated pneumonia, meningitis, septicemia, urinary tract infections and implant associated infections which might be explained by its high potential for biofilm production conferring outstanding antibiotic resistance, survival properties and increased virulence.[7,8]

Keeping these facts in mind, the present study was undertaken with the aims and objectives to detect biofilm production and its association with MDR among the clinical isolates of Acinetobacter in our hospital.

Materials and Methods

This prospective study was conducted at Yenepoya medical college, Mangaluru from June 2015 to December 2015.

The Acinetobacter species which were preserved at 4°c in nutrient agar were inoculated in trypticase soya broth and incubated at 37°c overnight for revival. The inoculum was taken from broth and inoculated on MacConkey agar for confirmation of viability and identification. After 24hrs of incubation at 37°c for 24hrs, NLF colonies were picked from MacConkey

plates and further processed by biochemical tests for identification of Acinetobacter species as per standard protocol using standard laboratory procedures. In this study Tube Method was used for Biofilm.[9]

The organisms isolated from each MacConkey plate were inoculated in 10 mL of trypticase soy broth with 1% glucose. Broths were incubated at 37ºC for 24 hrs. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. The fixation of biofilm was done with methanol (98%). The methanol was kept in tubes for 15-20 minutes at room temperature, after methanol was discarded and tubes were allowed to dry at room temperature. The tubes were incubated at room temperature for 5-10 minutes. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilm formed was scored as 1-weak/none, 2-moderate and 3-high/strong. Biofilms were also detected by spectrometry and OD reading was taken. The experiment was performed in duplicate, results recorded and mean OD value calculated.[9]

Antimicrobial sensitivity testing was done for the isolates obtained from blood, urine, tracheal aspirate by Kirby –Bauer disc diffusion Method and results interpreted as per CLSI guidelines.

The isolated organisms were subjected to Antibiotic susceptibility testing by Kirby Bauer Disk diffusion technique on Muller-Hinton agar plates (Hi-Media laboratories Pvt. Ltd., Mumbai) as per standard CLSI guidelines, combined with institutional antibiotic policy.

The antibiotic disks and their strength used for testing are as below.

Ampicillin-10µg   Ciprofloxacin-5µg
Amikacin-30µg   Chloremphenicol-30µg
Aztreonam-30µg   Co-trimoxazole-
1.25/23.75µg
Colistin-50 µg
Cefipime- 30 µg   Imipenem-10µg
Cefuroxime-30µg   Norfloxacin-10µg
Ceftriaxone-30µ   Piperacillin-100µg
Ceftazidime-30µg   Piperacillin-Tazobactam-
100/10 µg
Tetracycline-30 µg

Post-incubation, the zones of inhibition were measured and interpreted according to CLSI criteria.

Results

Table 1: Classification of biofilm formation by spectrophotometry (Tube method)

Mean OD value	Total[43]	Biofilm formation
<0.120	17	Non –biofilm
0.120-0.240	10	Moderate
>0.240	16	Strong

Table 2: Comparative chart of biofilm production in MDR vs NON-MDR isolates

Mean OD value	Biofilm formation	MDR	NON-MDR	Total
<0.120	Non	10	7	17
0.120-0.240	Moderate	7	3	10
>0.240	Strong	12	4	16
Total		29	14	43

A total of 26 (60%) of the 43 isolates of Acinetobacter showed biofilm production. The test was considered positive when there was an adherent layer of stained material on the inner side of the tubes. Isolates which showed stained material only at the liquid-air interface were considered negative (Fig. 3)

Out of 43 isolates of acinetobacter, 29 (67.4%) were multidrug resistant. Among MDR strains, 19 (65.5%) were biofilm producers and 10(34.4%) were non producers. Out of 14 Non-MDR strains, 7(50%) were biofilm producers and 7 (50%) were non producers, making it as 60.5% isolates producing biofilms. (Fig. 2) These findings are in concordance with the study conducted by Rodriguez B et al[8] wherein 63% of the 92 clinical isolates of Acinetobacter formed biofilm.

Acinetobacter presents a global medical challenge in causing opportunistic infections because of its ability to colonize and persist in the hospital environment, therefore a significant percentage of patients are at increased risk of being infected with biofilm producing isolates. It is also among the most common causes of device-related nosocomial infection because the organism is able to resist physical and chemical disinfection, often by forming a biofilm.[10,11]

In our study, biofilm producing *Acinetobacter* isolates showed highest sensitivity to colistin (100%) followed by imipenem (73.70%) and least sensitivie to ampicillin (0%) followed by ciprofloxacin (3.8%).

Sensitivity to aztreonam (19% vs 29.4%), amikacin (23.7% vs 35.29%), cefipime (31.20% vs 41.10%), ceftazidime (13% vs 23%), ciprofloxacin (3.8% vs

11.7%), chloramphenicol (29.40% vs 15.30%), pip-tazobactam (34.60% vs 41.10%), tetracycline (23.70% vs 29.40%) and Imipenem (73.70% vs 82.30%) were comparatively lower among biofilm producing acinetobacter than non-producing isolates. Sensitivity pattern of biofilm producing and nonproducing isolates of *Acinetobacter* are shown in Fig. 1.

Biofilm producing Acinetobacter showed > 70% resistance to aminoglycoside, fluoroquinolone and B-

lactam group of antibiotics. This finding is in concordance with the study done by Gurung J et al.[3]

Out of 26 biofilm producing isolates, 16 were strong producers and 10 were moderate producers graded according to the optical density reading taken by spectrometry.
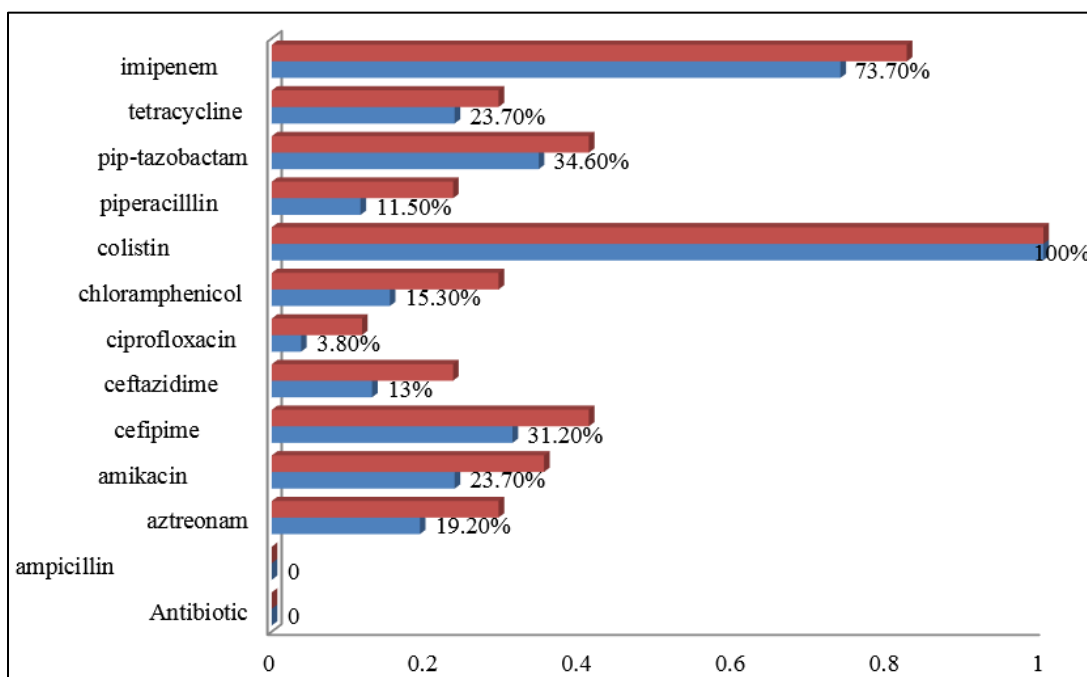

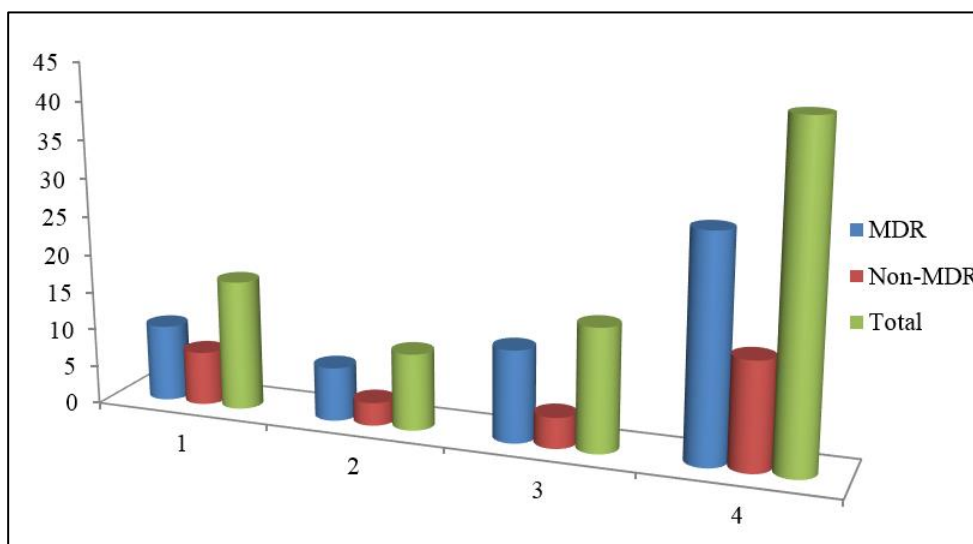**Fig. 1: Antibiotic sensitivity pattern of biofilm and non-biofilm isolates**

Fig. 2: Comparison of MDR and NON-MDR isolates

Fig. 3: Tube method of biofilm production-A; Negative control; B: Test; C: Positive control

Discussion

The higher rates of device-related infections are attributed to biofilm production by Acinetobacter which helps in persistent colonization.[12] The resistance develops by various methods like restricted penetration of antibiotics into biofilms, decreased growth rate and expression of resistance genes.[13] These isolates also account for the main epidemic clusters detected. Moreover, the MDR pattern can be transferred to other organisms that initially do not show such resistance. This emphasizes the importance of further research to develop treatments against Acinetobacter infections.

According to our study, there is positive association between biofilm positivity and multiple drug resistance. Compared to non-biofilm producers our study detected significantly higher resistance to antibiotics like amikacin, ciprofloxacin, aztreonam, piperacillin-tazobactam and imipenem. The potential ability of Acinetobacter to form biofilms could explain this outstanding antibiotic resistance.

The tube adherence assay followed in our study is very simple to perform and a reliable test used as general screening method for detection of biofilm producing organisms. The study conducted by Rewatkar A.R et al[5] concludes that Tube method is more qualitative and reliable method as compared to Congo Red Assay (CRA), moreso for strongly biofilm producing isolates. The drawback of the tube test is that its difficult to discriminate between weak and biofilm negative isolates due to variability in observed results by different observers.

Conclusion

Overall, the present study demonstrated a high propensity among the clinical isolates of Acinetobacter to form biofilm and there was a significant association

of biofilms with multiple drug resistance. The antibiotic susceptibility pattern as seen in individual hospital could help us formulate antibiotic policy, such as early aggressive antibiotic prophylaxis/therapy and chronic suppressive therapy that reduces biofilm production in the context of device-related infections. A greater understanding of the nature of biofilm and their role in serious infections will facilitate the development of more effective therapeutics and prevention against the biofilm-related infections that are superior to the current antibiotic treatment. Novel treatment strategies such as phage therapy, quorum-sensing inhibition and induced biofilm-dispersion have been documented in the literature.[14] Further research should be done in this field to provide us with the vital knowledge to combat this real threat.

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