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Biofilm production and multidrug resistance in clinical isolates of Acinetobacter baumannii in a tertiary care hospital

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I. INTRODUCTION

Biofilms are structured layer of bacterial communities' adherent to abiotic or biotic surfaces enclosed within a self-produced exopolysaccharide matrix (1). Bacteria producing biofilms are responsible for antibiotic resistance due to restricted penetration of antibiotics into biofilm and expression of resistant genes. In general, there is very scant literature available in Indian subcontinent on indiscriminate use of antimicrobial agents. The indwelling Medical Devices (IMDs) are most vulnerable to biofilm producing microbial colonizers and the superimposed complex nature of bacteria in biofilms colonizing IMDs, have resulted in phenomenon of resistant Device Related Infections (DRIs) (2).

Acinetobacter baumannii is Gram negative aerobic coccobacilli that are ubiquitous in nature and cause a variety of opportunistic nosocomial infections. A. baumannii is generally regarded as the second most common pathogen after Pseudomonas aeruginosa among the nosocomial, aerobic, non-fermentative, Gram negative bacilli pathogens which causes nosocomial pneumonia, bacteremia, meningitis, and urinary tract infection. It is the most common cause of device related nosocomial infection which is caused when the organism is able to resist any chemical or physical disinfectant by forming a biofilm e.g. vascular catheters, cerebrospinal fluid shunts, foley catheters etc. Multidrug-resistant Acinetobacter baumannii has been reported worldwide and is now recognized as one of the most difficult healthcare-associated infections to control and to treat (3). 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 A. baumannii in this tertiary care hospital.

II. MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, on different isolates from various clinical samples of IPD and OPD patients of all age groups after the clearance from Institutional Ethical Committee of research committee. A total of 74 isolates were screened and were subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method on Mueller Hinton Agar (MHA) and the zones were interpreted as per CLSI guidelines (4,5). The device related isolates which were Multi Drug Resistant (MDR) in nature were then further tested for biofilm production by the following enumerated methods:

Tube Adherence Method (TA)
The quantitative assay for Biofilm formation was performed according to the method described by (6). The test tubes were filled with 3 ml of Brain Heart Infusion medium (Himedia) which were inoculated with a loop full of a pure culture of a strains of *A. baumanii* were grown overnight from blood agar plate. After 48 hours of incubation at 37˚ C, the content of each tube was decanted. The tubes were then stained with 0.1% Crystal violet for 8 min. Then the tubes were washed with phosphate buffer saline pH 7.2 for 5 min. A positive result was indicated by the presence of an adherent film of stained material. The liquid-air interface alone was not regarded as indicative of slime production. Tubes containing BHI only were included in the test as negative controls.

Microtiter Plate Method (MTP)
Organisms were isolated from fresh agar plates and were inoculated in Brain Heart infusion Broth for 24 hours. The cultures were diluted 1:100 with fresh BHI broth. Wells of a sterile 96-well flat bottomed plastic tissue culture plate (Genaxy) were filled with 200 µL of bacterial suspension in brain heart infusion (BHI) broth and were incubated at 37˚ C for 24 hours. Negative control wells contained broth only. The plates were covered and incubated aerobically for 24 hours at 37˚ C. Then the content of each well were washed three times with 200 µL of PBS with pH 7.2 to remove non adherent bacteria. Biofilm formed by bacteria adherent to the wells were fixed with 2% sodium acetate and stained by 0.1% crystal

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violet. Excess stain was washed by demonized water and later air dried. The optical density (O.D.) of each well was measured at 570 nm using ELISA reader. The experiment was done in triplicates (7).

For the purpose of comparative analysis of test results, the adherence capabilities of the test strains were classified according to (8). Strains were classified as follows:

OD value of Biofilm Formation Microtiter plate Method	
Less than 0.120	Non Biofilm producer
In the range of 0.120 – 0.240	Moderate Biofilm Producer
Greater than 0.240	Strong Biofilm producer

III. RESULTS

The microbial strains were isolated from various indwelling devices, which were mostly left inside the body to maintain drainage, prevent obstruction, or provide a route for administration of food or drugs. The various indwelling devices from which organisms were isolated were central venous catheter, urinary catheter, central venous catheter, endotracheal tubes, tracheostomy tubes and chest drain tube tips. A total of 74 isolates were screened. Maximum number of *A. baumannii* were isolated from endotracheal tips (38) followed by tracheostomy tube tips which were 29 isolates in number. Only 4 isolates of *A. baumanii* were isolated from Urinary catheter tips. Antibiotic sensitivity testing showed that doripenem and tigecycline were the sensitive drugs towards biofilm producing strains with 23.08 and 65.38% sensitivity.

Antibiogram of A. baumannii

Antibiotics	Percentage sensitivity of Non Biofilm Producers	Percentage sensitivity of Biofilm Producers
Cefotaxime	0	0
Ceftazidime	0	0
Cefipime	0	0
Pippercillin+Tazobactam	42.86	5.77
Ticarcillin+Clavulinic acid	0	0
Ampicillin+sulbactam	0	0
Imipenem	66.67	23.08
Meropenem	23.81	13.46
Gentamycin	42.86	7.69
Amikacin	42.86	7.69
Ciprofloxacin	0.00	0.00
Cotrimoxazole	0.00	0.00
Chloramphenicol	0.00	0.00
Tigecycline	33.33	26.92
Doripenem	76.19	65.38
Polymyxin – B	95.24	92.31
Colistin	76.19	73.08

Biofilm production by various isolates of A. baumanii

Type of biofilm producers	Tube Adherence method	Microtiter plate method
Strong biofilm producers	6	7
Moderate biofilm producers	34	49
Non biofilm producers	34	18
Total	74	74

Imipenem and meropenem had 23.08% and 13.46% sensitivity against biofilm producing *A. baumanii*. Gentamycin and amikacin showed similar sensitivity of 7.69% towards biofilm producing strains. Tube adherence method showed total 6 isolates as strong biofilm producers whereas microtiter plate method showed 7 isolates as strong biofilm producers. A total of 49 strains of moderate biofilm producers were detected by microtiter plate method and 34 strains were moderate biofilm producers by tube adherence method.

IV. DISCUSSION

Acinetobacter baumannii infections present a global medical challenge. This class of non-fermenters has come out to be the opportunistic pathogens that are able to successfully colonize and persist in the hospital environment. Multidrug-resistance in *Acinetobacter baumannii* bacterial strains has been reported worldwide and is now considered as one of the most difficult healthcare-associated infections to control and treat. In the current study the maximum isolation was from endotracheal tube tips which were 51.34% which was similar to the study done by Sangitha *et al* 2014 (10) who

also reported the maximum isolation from endotracheal tube tips. The highest isolation from the devices this study may be co related with the origin of *A. baumanii* isolates from the skin of patients or healthcare workers, tap water to which entry ports are exposed, or other sources in the environment.

The total biofilm producing percentage of *A. baumanii* came out to be 74.67% which was quite high as compared to the previous reported studies done by Dheepa *et al* 2011 (9) who reported 60% of biofilm production.

Imipenem resistance came out to be 76.92% in the current study where as a study done in Puducherry India showed 100% resistance to imipenem (). In the current study sensitivity of different drugs was also calculated imipenem was 23.08% sensitive, meropenem 13.46%, pipercillin 5.77%, gentamycin 7.69% and amikacin was 7.69% sensitive where as in the study done by Dheepa *et al* 2011 who showed that efficacy of imipenem was 46%, meropenem 32%, pipercillin 28%, gentamycin 28% and amikacin 7.69% was sensitive. In *A. baumannii* polymyxin B showed and colistin were 92.31% and 73.61% sensitive. Similar result was also shown by Bose *et al*. (2015) (11) who showed that they were the drug of choice for the treatment of biofilm producing *A. baumannii.*

V. CONCLUSION

At the end in conclusion, in-vitro antimicrobial susceptibility of *A. baumannii* isolates in various geographical areas are necessary in order to generate continuous surveillance of the data useful for the empirical antimicrobial treatment of patients with infections which are likely to be caused by this pathogen. The current investigation shows simultaneous resistance to antimicrobial agents and biofilm production which represents a severe threat in the treatment of hospitalized patients. The high rate of *in vitro* antibiotic resistance of the *A. baumanii* strains indicate the importance of controlled antibiotic usage and appliance of hospital infection control measures.

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