

Antibiotic Resistance and Biofilm Formation in Gram Negative Bacteria Isolated From Endotracheal Tubes in Intensive Care Unit of a Tertiary Care Hospital



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ABSTRACT: Biofilm formation has been found to play a central role in many human infections in which antibiotic resistance is common. Bacteria can colonize and form biofilms on almost any surface, including natural and synthetic surfaces. The present study was conducted with an aim to detect biofilm formation in ET tubes/ ET secretions received from ICU patients by microtiter plate method. In the present study a total of 85 isolates were obtained out of 400 samples. Among 85 isolates of gram-negative bacteria, maximum number of isolates were of *K. pneumoniae* (41.17%) followed by *A. baumannii* (40%), *P. aeruginosa* (10.5%), *E. coli* (5.88%) and *B. cepacia* (2.35%). All isolates were tested for antimicrobial sensitivity/resistance by Vitek 2 Compact system. All the isolates were further tested for biofilm production by microtiter plate method and optical density (OD value) was measured. Biofilm production was distributed by strong, medium, and weak. It is concluded that stringent hygiene protocols, routine tube changes and the use of antimicrobial coatings are crucial for prevention of biofilm development in ICU settings.

KEYWORDS: Biofilms, Endotracheal tubes, ET secretions *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli*

INTRODUCTION

Biofilms play an important role in the pathogenesis of device-related infections and drug resistance. Microorganisms attach to synthetic surfaces, multiply and develop biofilms characterized by the generation of an extracellular polymeric substance or matrix that has been well documented with scanning electron microscopy (SEM) studies.^[1] Ventilator associated Pneumonia (VAP) develops by direct entry of bacteria to lower respiratory tract, which may be innate flora of oropharynx or those present in the hospital via micro aspiration, which can occur during intubation itself, development of a biofilm overloaded with bacteria (typically Gram-negative bacteria and fungal species) within the endotracheal tube, pooling and trickling of secretions around the cuff and impairment of mucociliary clearance (disrupting the cough reflex, thus promoting the accumulation of tracheo-bronchial secretions and increasing the risk of pneumonia).^[2,3] In addition, the insertion of an ETT could produce injury and inoculate endogenous oropharyngeal bacteria in the low airway tract.^[4] Formation of biofilm on the surface of Endotracheal tube (ETT) is an almost universal phenomenon and it has been strongly related to the pathogenesis VAP. Due to the role of ETTs in the pathophysiological development of VAP, some authors suggest that it should be renamed ETT-associated pneumonia.^[5] Various phenotypic methods are available to detect biofilm formation such as Congo red agar method, microtitre plate method, electron microscopy, confocal scanning microscopy and bioluminescence analysis. Biofilm formation in microtiter plates is the most commonly used method to grow and study biofilm. This simple design is very popular due to its high-throughput screening capacities, low cost, and easy handling. Detection of biofilm-related genes using PCR methods has been increasingly used, but may not be feasible as a routine diagnosis in resource-limited settings.^[6]

The current study aimed to study the presence of biofilm formation by various Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* isolated from endotracheal tubes and endotracheal secretions obtained from mechanically ventilated patients using microtitre plate method.

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MATERIALS AND METHODS

This observational study was conducted from August 2022 to January 2023 in Department of Microbiology, Adesh Institute of Medical sciences and Research, Bathinda. Approval of Institutional Research Committee and Ethics Committee for Biomedical and Health Research Adesh University was taken before start of the study. A total of 400 samples of ET tubes/ ET secretions were received in Bacteriology lab from patients admitted in various ICU/s of AIMSR.

The sample size was calculated using the formula, $n = z^2 p (1-p)/d^2$, where n is the sample size, z is the statistic corresponding to the level of confidence (1.96), p is the prevalence, and d is the allowable error i.e 10% of p.[6] In the present study, the estimation of sample size was done using the prevalence value p= 50% (0.5) based on previous study by Badia et al 2021. Based on this calculation, the n value was estimated and obtained to be 400 in the present study. [7]

Inclusion Criteria: ET tube /ET secretions samples showing pure as well as significant bacterial growth of $\geq 10^3$ CFU/ ml were included in the study.

Exclusion Criteria: ET tube /ET secretions samples showing mixed bacterial or fungal growth were excluded.

All the Endotracheal tube/ ET secretion samples received in Bacteriology laboratory were processed as per standard microbiological procedures. [8] Samples were inoculated on Blood agar and MacConkey agar by streak culture (Semi Quantitative method). [9] The plates were incubated at 37°C for 18-24 hrs. Gram staining of the specimens was performed to see the presence of polymorphonuclear leucocytes, gram positive/ gram negative bacteria or budding yeast cells. Ziehl Neelsen staining was performed to see the presence of acid-fast bacilli and KOH mount was prepared to check the presence of any fungal elements. The isolated organisms were identified on the basis of gram staining morphology, colony characters and biochemical tests. [10,11]

Antibiotic susceptibility testing was done by Vitek 2 compact System. [12] Further identification of biofilm formation by the gram negative bacterial isolates was performed by microtiter plate method and optical density (OD value) was measured. Biofilm production was distributed according to the OD value as strong (OD value ≥ 0.745), medium (OD value between 0.496 and 0.744), and weak (OD value between 0.248 and 0.496). [13]

RESULTS

A total of 400 ET samples were received and out of total 400 samples, 85 (21.5 %) samples showed significant growth ($\geq 10^3$ CFU/ml) and no growth was observed in 315 (78.5 %) samples after incubation period of 24-48 hrs.

Out of total 85 isolates, all isolates were gram negative bacteria and no gram-positive bacteria was isolated. Among 85 isolates of gram-negative bacteria, maximum number of isolates were of *A.baumannii* (40%) followed by *K. pneumoniae* (41.17%), *P. aeruginosa* (10.5%), *E. coli*(5.88%) and *B.cepacia* (2.35%). Out of 85 isolates, 63 (74.2%) isolates were obtained from males and 22 (25.8%) from females. Maximum isolates (48.23%) were obtained from the age group of 61-80 years, followed by age group of 41-60, 21-40 and 0-20 years. Antibiotic resistance profile of Gram negative bacteria isolated in the study is shown in Table 1.

Table 1: Antibiotic resistance profile of Gram negative bacteria (N=85)

Name of Antibiotic	<i>K.pneumoniae</i> (n=35)	<i>E.coli</i> (n=5)	<i>A. baumannii</i> (n=34)	<i>P. aeruginosa</i> (n=9)	<i>B. cepacia</i> (n=2)
Cefepime	27 (77.14%)	5 (100%)	32(94.11%)	5 (55.55%)	NT
Cefuroxime	33 (94.28%)	5 (100%)	NT	NT	NT
Ceftriaxone	33 (94.28%)	5 (100%)	NT	NT	NT
Ceftazidime	NT	NT	34(100%)	5 (55.55%)	2(100%)
Amoxicillin+ Clavulanic acid	30 (85.71%)	5 (100%)	NT	NT	NT
Cefoperazone+Sulbactam	30 (85.71%)	2 (40%)	32 (94.11%)	3 (33.33%)	NT
Piperacillin+Tazobactam	27 (77.14%)	NIL (0%)	34 (100%)	2 (22.22%)	NT
Ciprofloxacin	35 (100%)	5 (100%)	33 (97.05%)	6 (66.66%)	NT
Levofloxacin	NT	NT	33 (97.05%)	4 (44.44)	NIL (0%)
Gentamicin	28 (80%)	NIL (0%)	32 (94.11%)	5 (55.55%)	NT
Amikacin	NIL (0%)	NIL (0%)	30 (88.23%)	2 (22.22%)	NT
Imipenem	29 (82.85%)	3 (60%)	30 (88.23%)	4 (44.44%)	NT

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Meropenem	27 (77.14%)	2 (40%)	32(94.11%)	3 (33.33%)	NIL(0%)
Cotrimoxazole	27 (77.14%)	5 (100%)	30 (88.23%)	NT	NIL(0%)
Minocycline	NT	NT	24 (70.57%)	NT	NIL (0%)

NT- Not tested (as per CLSI guidelines)

In the present study, more strong biofilm producers were of *K. pneumoniae* followed by *A.baumannii* (20%, and 8.9%) respectively, medium biofilm producers were more of *A.baumannii* as compared to *K. pneumoniae* (85.2% and 74.3%) respectively, whereas all isolates of *P. aeruginosa*, *E.coli*, *B. cepacia* were weak biofilm producers as shown in Table 2.

Table 2: Distribution of isolates according to strong, medium and weak biofilm formation

Name of the organism	Strong positive	Medium positive	Weak positive
<i>A. baumannii</i>	03 (8.9%)	29 (85.2%)	02 (5.9%)
<i>K. pneumoniae</i>	07 (20%)	26 (74.3%)	02 (5.7%)
<i>E. coli</i>	Nil	Nil	05 (100%)
<i>P. aeruginosa</i>	Nil	Nil	09 (100%)
<i>B. cepacia</i>	Nil	Nil	02 (100%)

DISCUSSION

In the present study, the total isolates obtained were 85 gram negative bacilli (21.25%). The prevalence shows similarity with the studies done by Uppe et al, Rewdiwala et al, Dargahi et al who reported prevalence of 25%, 22%, and 20% respectively. [13,14,15]

In the present study, out of 85 biofilm producing isolates, 74.2 % isolates were obtained from males and 25.8 % from females. Similar results have been reported by various other studies- Rewdiwala et al, Kaur et al, Dicenu et al and Bhat et al. [14,16,17,18]

In the present study maximum isolates were obtained from age group 61-80 years (48.23%). Baidya et al and Uppeetal also reported similar findings (44.4% and 53.3%) respectively , that the highest number of isolates were obtained from age group of 61-80 years. [7, 13]

In the present study, *A. baumannii* and *K. pneumoniae* were the most common isolates followed by *P. aeruginosa* and *E. coli*. The results of this study correlates with various other studies done by Uppe et al, Rewdiwala et al, Kaur et al, Sharma et al, [13,14, 16,19]

A. baumannii, *K.pneumoniae* and *P. aeruginosa* showed multiple drug resistance which in concordance with various other studies done by Baidya et al, Sharma et al, Amin et al, Tomar et al and Natham et al. [7,19,20,21,22] . In the present study, *B. cepacia* isolates showed susceptibility towards levofloxacin, minocycline, meropenem and cotrimoxazole. In a study conducted by Dutta et al, Shukla et al and Siddiqui et al, *B. cepacia* showed maximum susceptibility towards minocycline and cotrimoxazole. [23,24,25] Kady et al reported *B. cepacia* isolates to be 100% susceptible to meropenem, ceftazidime, cotrimoxazole, and minocycline. [26] In the present study, 23.5% were weak biofilm producers, 64.5% were medium producers and 12 % were strong biofilm producers. Baidya et al also reported that 25.3% were weak, 62.5% were medium and 12.2% were strong biofilm producers. [7]

Rewdiwala et al reported that, 22.5% wereweak biofilm producers, 64.7% were medium producers and 12.8% werestrong biofilm producers. [14] Kaur et al (2017) reported that 32,4% were weak biofilm producers, 58.8% were medium biofilm producers and 8.8% were strong biofilm producers [16] The results obtained in the present study are concordant to Baidya et al, Rewdiwala et al and Kaur et al.

CONCLUSION

The present study, showed that all the pathogens isolated in this study possessed capability to produce biofilms. Therefore, it is necessary to establish standard guidelines on the use of indwelling devices especially ETTs in all units of the hospital environment with a view to prevent nosocomial infections in patients related to the devices. Preventivemeasures, including stringent hygiene,

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protocols, routine tube changes and the use of antimicrobial coatings, are crucial for mitigating the risk of biofilm development. Continued research and innovation are necessary to develop more effective strategies for biofilm prevention and management within ET tubes, with the goal of enhancing patient safety and improving clinical outcomes.

Ethical approval- The study was approved by the institutional Ethics Committee for Biomedical and Health Research.

CONFLICT OF INTEREST- None

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