Biofilm producer multi drug resistance alert bugs in ventilator associated pneumonia patients. Threat to antibiotic era and future concern
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Abstract

Background
Emerging threat of drug resistance among Bacteria causing ventilator-associated pneumonia (VAP) has resulted in higher hospital costs, longer hospital stays, and increased hospital mortality. Biofilms in the endotracheal tube of ventilated patients act as protective shield from host immunity for bacterial growth and emerge them as multidrug resistant.

Aim
To know the prevalence of various bacterial isolates causing VAP, ability to form biofilm and their antibiotic susceptibility pattern.

Material & Methods
This study was conducted in the department of Microbiology in collaboration with the Respiratory Medicine department for a period of one year (November 2018-19). Endotracheal aspirate (ETA) along with 1 cm tube tip from clinically confirmed VAP patients were processed as per the standard microbiological procedure for the detection of bacterial biofilm formation and their antimicrobial resistance pattern. Statistical Analysis: Data was statistically evaluated using SPSS-PC-20 version. ‘P’ value less than 0.05, considered statistically significant.

Results
72 patients with CPIS score > 6 were clinically confirmed as VAP. Various Bacteria isolated were Klebsiella pneumoniae in 52 (53%), Escherichia coli 16 (16.3%), Pseudomonas aeruginosa 14 (14.2%), Acinetobacter spp. 8 (8.1%), Proteus mirabilis 6 (6.1%) and Pseudomonas luteola 2 (2%). All bacterial isolates were processed for their ability to form biofilm, 86 (87.7%) were biofilm producers (BFP) while 12 (12.2%) were biofilm non-producers (BFNP).

Conclusion
Bacterial etiology, prolonged intubation, biofilm formation, and drug resistance have ramification on outcome of VAP.

Keywords: Ventilator associated pneumonia (VAP), CPIS score, Biofilm formation, Tissue culture plate method (TCP), Antimicrobial drug resistance (AMR)
Background
Endotracheal tubes (ETT) act as a reservoir for multidrug resistant (MDR) superbugs, providing them a surface to adhere and to produce biofilm among mechanically ventilated patients.  

The rationale behind this study was to unravel the relation of biofilm production with emergence of multidrug resistant superbugs among ventilator associated pneumonia (VAP) patients causing alarming rise in patients’ mortality in our healthcare settings.

Material and Methods
This study was carried out in the department of Microbiology in collaboration with Respiratory Medicine department, for a period of one year (November 2018-2019).

72 Endotracheal aspirates (ETA) along with 1 cm tube tip, from clinically confirmed VAP patients were collected after extubation or during changing the ETT (Romson’s cuffed non-coated with antibiotics ETT was taken), processed as per the standard microbiological procedure for detection of bacterial biofilm formation and their antimicrobial resistance pattern.

The study was approved by the Institutional Ethical Committee. (Letter no. 1359/UPUMS/Dean/2020-21 dated 28th October 2020).

Inclusion Criteria
Patients >18 years age admitted in ICU, mechanical ventilation support > 48 hours, having clinical symptoms and radiological changes in lungs categorized as VAP according to CPIS (Clinical Pulmonary Infection Score). (Table 1)

Exclusion Criteria
Patients under 18 years of age, those having pneumonia on admission or during the first 48 hours of mechanical ventilation, patients have clinical symptoms after intubation but failed to meet the criteria of CPIS score.

Clinical Selection Criteria (CPIS SCORE) for VAP
It is a scoring system, based on six parameters (clinical, radiological, and microbiological) with each parameter given a score scale ranging from 0 to 2 each for fever, leukocyte count, quantity and purulence of tracheal secretions, oxygenation status, chest radiographic abnormality, and the result of endotracheal aspirate culture and gram stain. The maximum score that can be obtained is 12 and a score > 6 is diagnostic of VAP. The specimen was then collected by the critical care physician on duty using an aseptic technique.

Sample Processing
Quality of the aspirate received was ascertained by counting the composite quality score of a gram stained smear and observing it under high power microscope for presence of Polymorphonuclear neutrophils (PMNs) (more than 25 per high-power field) and absence of epithelial cell.

The sample was streaked using 1μL calibrated loop on Blood agar and MacConkey’s agar plate and incubated overnight at 370C. The cut-off criterion was 105 CFU/mL for ETA to differentiate between pathogens and contaminants. Isolates in pure growth or mixture of two organisms at quantitative threshold were considered significant and processed. Bacterial identification and antibiotic susceptibility testing were done using automated VITEK® 2 system. Biofilm detection was done by microtiter Tissue culture plate method. Biofilm production Interpretation was done according to Stepanovic et al criteria. Optical density between ranges 2-4 are moderate biofilm producers and above 4 are strong biofilm producers.

Statistical analysis: Data was statistically evaluated using SPSS-PC-20 version. Chi-square test and paired ANOVA test were used. P value less than 0.05 were considered statistically significant.
Table 1: Clinical Pulmonary Infection Score

<table>
<thead>
<tr>
<th>Assessed parameter</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>≥36.5°C and ≤ 38.4°C</td>
<td>≥38.5°C and ≤ 38.9°C</td>
<td>≥39°C or ≤ 36°C</td>
</tr>
<tr>
<td>Leukocytes in blood (cells/mm³)</td>
<td>4,000-11,000</td>
<td>&lt;4000 or &gt; 11,000</td>
<td>&lt;4000 or &gt; 11,000 + band forms ≥50%</td>
</tr>
<tr>
<td>Tracheal secretions</td>
<td>Rare</td>
<td>Non-purulent</td>
<td>Abundant + purulent</td>
</tr>
<tr>
<td>Oxygenation status PaO₂/FiO₂ mm Hg</td>
<td>&gt;240 with ARDS</td>
<td>-</td>
<td>≤240 and absence of ARDS</td>
</tr>
<tr>
<td>Chest radiograph</td>
<td>No infiltrate</td>
<td>Diffuse/patchy infiltrate</td>
<td>Localized infiltrate</td>
</tr>
<tr>
<td>Culture results (Endotracheal aspirate)</td>
<td>No or mild growth</td>
<td>Moderate or florid growth of pathogenic bacteria</td>
<td>Moderate or florid growth and pathogen consistent with gram stain</td>
</tr>
</tbody>
</table>

Results

72 patients with CPIS score > 6 were clinically confirmed as VAP. Male participants were preponderant accounting for 55.5% (n = 40) while female participants summed to 44.4% (n=32), with male to female sex ratio being 1.25. Neurological complications 24 (33.3%) was the main cause for ICU admission among VAP cases, followed by organo-phosphorous poisoning 18 (25%), diabetes and cardiac pathology were seen in 22.2% of cases. 4 (5.6%) cases had primary respiratory etiology.

Most of the ETA showed monomicrobial growth, 46 (63.8%) while only 26 (36.1%) were polymicrobial in nature yielding a total of 98 isolates. Mono-microbial etiology was associated with 88.2% cases of early VAP and 42.1% cases of late onset VAP, whereas polymicrobial etiology was seen in 11.8% cases of early onset and 57.8% cases of late onset VAP, which was statistically significant (P < 0.001). (Table 2)

Most common bacterial isolates, isolated were Klebsiella Pneumoniae: 52 (53%), followed by Escherichia Coli: 16 (16.3%), Pseudomonas Aeruginosa: 14 (14.2%), Acinetobacter Spp.: 8 (8.1%), Proteus Mirabilis 6 (6.1%) and Pseudomonas Luteola: 2(2%). Out of 98 isolates, 86 (87.7%) were biofilm producers (BFP) and 12 (12.2%) were non-producers of biofilm (BFNP).

Major BFP were 52 isolates of Klebsiella Pneumoniae (60.4%), followed by 10 Pseudomonas Aeruginosa isolates (11.6%), 8 isolates of Acinetobacter species and Escherichia Coli each (9.3%), 6 isolates of Proteus Mirabilis (6.9%), 2 isolates of Pseudomonas Luteola (2.3%).

Out of 86 BFP, the majority isolates were “moderate” producers accounting for 67.4% (n = 58), followed by “strong” producers summing 32.5% (n=28).

For Klebsiella Pneumoniae, 18 isolates (20.9 %) were “strong” producers and 34 (39.5%) were “moderate” producers. For Escherichia Coli each (4.6%) were “moderate” and “strong” producers. 10 isolates (11.6%) of Pseudomonas Aeruginosa were only “moderate” producers. For Acinetobacter species 2 isolates (2.3 %) were “strong” producers and 6 (6.9%) were “moderate” producers. For Proteus Mirabilis 2 isolates (2.3 %) were “strong” producers and 4 (4.6%) were “moderate” producers. Both isolates (2.3%) of Pseudomonas Luteola were “strong” producers of biofilm. Biofilm producing isolates showed increased resistance to the majority of classes of antibiotics tested. (Table 3)
Table 2: Association of etiology with onset of VAP (Ventilator associated pneumonia)

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Early onset VAP (n=34)</th>
<th>Late onset VAP (n=38)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Monomicrobial</td>
<td>30</td>
<td>88.2</td>
<td>16</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>4</td>
<td>11.8</td>
<td>22</td>
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</table>

Table 3 Antibiotic resistance patterns among BFP (Biofilm producer) and BFNP (Biofilm non producer)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>BFNP</th>
<th>BFP (Strong + Moderate)</th>
</tr>
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<tbody>
<tr>
<td>Amikacin (AK)</td>
<td>2 (16.7%)</td>
<td>54 (62.8%)</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam (PTZ)</td>
<td>6 (50.0%)</td>
<td>66 (76.7%)</td>
</tr>
<tr>
<td>Cefoperazone Sulbactam (CFS)</td>
<td>4 (33.3%)</td>
<td>68 (79.1%)</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>2 (50.0%)</td>
<td>16 (80.0%)</td>
</tr>
<tr>
<td>Cefepime (CPM)</td>
<td>8 (66.7%)</td>
<td>70 (81.4%)</td>
</tr>
<tr>
<td>Meropenem (MRP)</td>
<td>4 (33.3%)</td>
<td>52 (60.5%)</td>
</tr>
<tr>
<td>Imipenem (IPM)</td>
<td>4 (33.3%)</td>
<td>62 (72.1%)</td>
</tr>
<tr>
<td>Cotrimoxazole (COT)</td>
<td>8 (100.0%)</td>
<td>56 (73.7%)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>8 (66.7%)</td>
<td>68 (79.1%)</td>
</tr>
<tr>
<td>Colistin (CL)</td>
<td>0</td>
<td>14 (16.3%)</td>
</tr>
</tbody>
</table>

**Discussion**

Biofilm formation by pathogens is posing a threat in infection control regardless of the ample choices of antibiotics available. While the indiscriminate use of broad-spectrum antibiotics has tapered down the choices of effective drugs to combat the so-called “superbugs,” the dreary reality is worrisome due to the spread of biofilm formation. The recent upsurge of biofilm production and drug resistance among such organisms has pulled back the era of medicine by decades as we have limited choices of antibiotics left. Early detection of biofilm formation can help modulate the treatment strategies and hence reduce mortality and morbidity.

In our study, VAP prevalence was 72%, much higher in comparison to other Indian studies by Gupta et al, Thakuria et al and Ranjan et al who reported 28.4%, 51%, and 57.14% respectively. This high prevalence in the present study could be due to associated comorbidities and shorter study period.

In our study, 63.8% and 36.1% cases had monomicrobial and polymicrobial etiology respectively. Charles et al and Kant et al reported predominance of monomicrobial etiology in 72.8% and 51.92% respectively. Patil et al reported predominance of polymicrobial in 55.4% cases.

47.2% cases were of early onset (<4 days of hospitalization) and 52.7% of late onset (>4 days of hospitalization) in our study in concordance with Dey et al and Goel et al. Our results were statistically significant (P <0.001). Maximum prevalence rate was of K. Pneumoniae (53%) similar to study by Hira et al, Alqurashi et al, Chidambaram et al who reported 36%, 31% and 69% respectively.

Ability of VAP bacterial isolates were assessed for in-vitro production of biofilm with emerging antimicrobial resistance. Out of 86 biofilm producers isolates, 67.4 % (n = 58) were “moderate” producers and 32.5% (n=28) were “strong” producers similar to Hassan et al, and Mathur et al who reported 22.7%, 14.47%, strong producers and 41%, 39.4% moderate producers. Major Biofilm producer was K. Pneumoniae (53%) followed by P. Aeruginosa.
Agarwal K. Biofilm producer multi drug resistance alert bugs in ventilator associated pneumonia patients. Threat to antibiotic era and future concern.

K. Pneumoniae was predominantly strong and moderate biofilm producer in 20.9% and 39.5%. Table 2 depicted biofilm producing isolates were found to be more resistant to multiple antibiotics groups similar to study by Hasan et al, 17 and Subramanian et al. 19

Hence prior antibiotic treatment and prolonged mechanical ventilation are important risk factors associated with development of multi drug resistance. Since biofilms develop slowly over a period of time, biofilm-related infections are diagnosed in the later course of the disease after the biofilm has been established. Hence treatment in an already biofilm positive case is less effective compared to removing the biofilm present on the ETT. Hence removal of ET tube in regular intervals might be considered with a proper choice of antimicrobial treatment or using ET tube coated with drugs/ biomaterials that discourage biofilm formation may be explored.

Conclusion

Biofilm forming bacteria in VAP patients have grave clinical outcomes due to multi drug resistance. These superbugs are hard to treat and have limited treatment therapy. It advocates the need for further research in framing convenient methods of Biofilm detection and alternative treatment therapy. Implementing bundle approach, infection controls practices and early diagnosis among ventilated patients is need of an hour.

References


