### Background

- **Klebsiella pneumoniae** is a frequently multidrug-resistant organism with a high propensity to produce biofilm.
- K. pneumoniae is the most common carbapenem-resistant Enterobacteriaceae (CRE), which have been categorized as an urgent threat by the CDC and is associated with high mortality rates up to 50%-6
- The relationship between **K. pneumoniae** biofilm formation and antimicrobial resistance has not been extensively described and data is limited:
  - Extensively drug resistant (XDR) **K. pneumoniae** urinary isolates from a rehabilitation center formed biofilm at higher rates than MDR strains and susceptible strains (91.1% vs. 67.5% vs. 70.8%, respectively).
  - Biofilm forming **K. pneumoniae** from a single-center hospital more likely produced ESBL versus non-biofilm formers (83.6% vs. 14.4%, p < 0.01)\(^7\)
  - **Biofilm** forming **K. pneumoniae** isolates from a single-center hospital outbreak were more likely to produce biofilm versus carbapenem susceptible isolates (p < 0.05)\(^8\)

### Methods

#### Isolate susceptibility

- Based on 2017 CLSI breakpoints, except for tigecycline, colistin, and fosfomycin as CLSI breakpoints were unavailability

#### Categorization for MDR and XDR was based on CDC/ECDC consensus\(^9\)

- **Multidrug-resistant (MDR):** non-susceptible to ≥ 1 agent in ≥ 3 out of 16 antimicrobial categories
- **Extensively drug-resistant (XDR):** non-susceptible to ≥ 1 agent in all but ≤ 2 out of 16 antimicrobial categories

#### The antimicrobial categories were collapsed to 9 groups below (Table 1)

#### Statistical analysis (SAS 9.2):

- Differences assessed with Chi-square or Fisher’s exact tests for dichotomous variables and student t-test for continuous variables
- Predictors of strong biofilm formation were identified with logistic regression

### Results

**Table 1. Prevalence of Antimicrobial Resistance According to Biofilm Formation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Cohort (n=93)</th>
<th>Weak Biofilm Formation (n=47)</th>
<th>Strong Biofilm Formation (n=46)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multidrug-resistant (MDR), n (%)</td>
<td>81 (87.1)</td>
<td>46 (97.9)</td>
<td>35 (76.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Extensively drug-resistant (XDR), n (%)</td>
<td>25 (26.9)</td>
<td>12 (25.5)</td>
<td>13 (28.3)</td>
<td>0.77</td>
</tr>
<tr>
<td>Number of Resistant Categories (n=16), Med</td>
<td>13</td>
<td>13</td>
<td>11.5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Antimicrobial Groups (n=9)**

| Penicillin / β-lactamase inhibitors, n (%) | 79 (84.9) | 46 (97.9) | 33 (71.7) | 0.0004 |
| Cephalosporins, n (%) | 82 (88.2) | 46 (97.9) | 36 (78.3) | 0.003 |
| Monobactams, n (%) | 73 (78.5) | 45 (95.7) | 28 (60.9) | <0.0001 |
| Carbapenems, n (%) | 70 (75.3) | 44 (93.6) | 26 (56.5) | <0.0001 |
| Protein Synthesis Inhibitors, n (%) | 80 (86.0) | 46 (97.9) | 34 (73.9) | 0.001 |
| Fluoroquinolones, n (%) | 73 (78.5) | 45 (95.7) | 28 (60.9) | <0.0001 |
| Folate pathway inhibitors, n (%) | 66 (71.0) | 37 (78.7) | 29 (63.0) | 0.09 |
| Fosfomycin, n (%) | 61 (65.6) | 29 (61.7) | 32 (69.6) | 0.42 |
| Colistin, n (%) | 11 (11.8) | 8 (17.0) | 3 (6.5) | 0.12 |

**Figure 1. Biofilm Categorization**

### References


### Conclusion / Discussion

- Previously published data describe resistant **K. pneumoniae** isolates to be associated with biofilm formation. However, our study comparatively:
  - Consists of diverse collections of isolates from multiple centers
  - Uses multivariate statistical analysis
  - Redefines biofilm quantification to overcome methodological limitations
  - Standardization of biofilm methods is imperative
  - Carbenem resistant **K. pneumoniae** were 91% less likely to form biofilm
  - The inverse relationship between biofilm formation and antibiotic resistance suggests there may be a trade-off for survival
  - Potential clinical impact: allows clinicians to better understand the optimal treatment approach for their patients if biofilm formation is present

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**Hypothesis / Objectives**

- **Hypothesis:** There is an association between specific **K. pneumoniae** antimicrobial resistance and biofilm formation

- **Objectives:**
  - Determine biofilm formation and antimicrobial susceptibility for each **K. pneumoniae** isolate
  - Identify if an association exists between **K. pneumoniae** biofilm formation and specific antibiotic resistance

### Methods

#### Organisms:

139 **K. pneumoniae** isolates were accessed from the CDC (n=66), BEI (n=36), ATCC (n=3), and patient isolates from Providence VA Medical Center and Rhode Island Hospital (n=34)

#### Biofilm Quantification:

- Modified Crystal Violet Method (OD\(_{570}\)\(^9\)

- **Biofilm formation** was defined by tertile cut-points\(^10\)

#### Figure 1. Biofilm Categorization

**K. pneumoniae** Isolates (n=139)

- **Weak biofilm formation**
  - OD\(_{570}\) > 0.16 (n=47 isolates)
- **Moderate biofilm formation**
  - 0.16 < OD\(_{570}\) < 0.59 (n=46 isolates)
- **Strong biofilm formation**
  - OD\(_{570}\) > 0.59 (n=46 isolates)

#### Final Analysis (n=93)

**Tertile Cut-Points**

- **Biofilm OD\(_{570}\) < 0.16 (n=47 isolates)**
- **Moderate Biofilm OD\(_{570}\) = 0.16 - 0.59 (n=46 isolates)**
- **Strong Biofilm OD\(_{570}\) > 0.59 (n=46 isolates)**

### Results

- 93 isolates remained upon removal of moderate biofilm formers
- MDR isolates were more common among weak biofilm formers (79.7%)
- XDR isolates were similar between groups (25.5% vs. 28.3% p=0.77)
- Resistance to penicillin/β-lactamase inhibitors, cephalosporins, monobactams, carbenapens, protein synthesis inhibitors, or fluoroquinolones were more common among weak biofilm formers (p=0.05)

#### Predictors regression model results:

- Carbenem resistance is inversely associated with strong biofilm
  - OR 0.09 (95% CI 0.02 – 0.33)

**Klebsiella pneumoniae** Biofilm Formation (n=93)

- **Optical Density (OD)**
  - OD = 0.0004
  - OD = 0.001
  - OD = 0.003
  - OD = 0.0001
  - OD = 0.0001

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