Similar virulence properties of infection and colonization associated *Pseudomonas aeruginosa*

Aditi,¹ Malini Shariff,¹,* Sunil K. Chhabra² and Mujeeb-ur Rahman³

**Abstract**

**Purpose.** *Pseudomonas aeruginosa* is one of the agents that are commonly implicated in nosocomial infections. However, it is also present as a commensal in various body sites of healthy persons, making the diagnosis of infection by culture difficult. A number of virulence factors expressed by the organism have been implicated in its pathogenicity. We undertook this study to identify the host and organism factors associated with infection.

**Methodology.** Pathogenic, colonizing and environmental isolates were tested for *apr, lasB*, the T3SS effector exoenzymes (*exoS, exoT, exoU* and *exoY*) and *toxA* genes, biofilm production and antimicrobial susceptibility. The isolates were further typed by RAPD.

**Results.** Eighty-seven isolates from 61 patients, including 11 environmental isolates, were obtained. None of the virulence factors were found to be significantly associated with infection, and nor was the antimicrobial susceptibility. The presence of the *exoU* gene and infection by MDR strains correlated significantly with the duration of hospital stay. Positivity for *exoS* and *exoU* genes was found to be strongly correlated with multi-drug resistance. *exoU* positivity correlated strongly with fluoroquinolone resistance. Sinks in the ward and intensive care unit were found to be a niche for XDR *P. aeruginosa*. Eighty-five isolates were typeable using the ERIC2 primer, showing 71 distinct RAPD patterns with >15% difference in UPGMA-generated dice coefficients.

**Conclusions.** *exoU* positivity is associated with severe disease, as evidenced by the longer duration of hospital stay of these patients. However, the presence of virulence factors or multi-drug resistance in the cultured strain should not prompt the administration of anti-pseudomonal chemotherapy.

**INTRODUCTION**

*Pseudomonas aeruginosa* is an opportunistic and nosocomial pathogen [1]. It commonly causes respiratory, urinary tract, bloodstream, skin and soft tissue infections [2]. It is also a colonizer in various body sites [3] and a contaminant in the hospital environment [4–6]. Colonization rates are known to increase significantly in hospitalized patients [7], including patients with chronic respiratory diseases [8]. Since *P. aeruginosa* may be present as a colonizer, isolation of the organism from clinical samples does not necessarily indicate the presence of infection. This presents a dilemma to the clinician – proceeding with anti-pseudomonal therapy may be unnecessary and harmful, while electing to withhold treatment may carry an unacceptably high risk of morbidity and mortality [9].

The organism expresses a multitude of virulence factors, which contribute to its pathogenicity. These include exotoxins, endotoxins, toxins secreted by protein secretion systems, phenazines and the ability to form biofilms among them. Though some of these have been associated with infection [10–13], their role in the establishment of human infection is uncertain. The type three secretion system (with its effector enzymes ExoS, ExoT, ExoU and ExoY) is a major virulence factor and has been associated with infection in some studies [13–15]. The relative virulence of its effector proteins is important, since clinical isolates of *P. aeruginosa* commonly fall into one of the following three phenotypic categories:

1. those that secrete ExoU and ExoT;
2. those that secrete ExoS and ExoY; and

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**Keywords:** *Pseudomonas aeruginosa*; infection; colonization; virulence; resistance; RAPD.

**Abbreviations:** ICU, intensive care unit; MDR, multi drug resistant; PDR, pan drug resistance; RAPD, random amplification of polymorphic DNA; UPGMA, unweighted paired group method with arithmetic mean.

One supplementary figure is available with the online Supplementary Material.
METHODS

Subjects and bacterial isolates
A prospective study was conducted over 15 months from January 2015 to March 2016 at a pulmonary diseases hospital, with approval from the Human Ethics Committee of the institute. In-patients with a positive culture for *Pseudomonas aeruginosa* were included in the study. Written informed consent was obtained from all patients.

Surveillance samples – nasal swabs, pharyngeal swabs, and urine and stool samples – were collected from patients at the time of admission and weekly thereafter until discharge or death. Other relevant clinical samples (sputum, tracheal and bronchial aspirates, blood, urine and pus) from these patients that were sent to the aerobic culture laboratory when clinically indicated were also included. Environmental samples and surveillance samples from healthcare workers were collected monthly from the ward and the intensive care unit (ICU).

The criteria defined by the National Nosocomial Infection Surveillance system were used to identify the presence of infection in patients who were positive for *P. aeruginosa* in culture [17]. The remaining patients with positive culture for *P. aeruginosa* were classified as colonized. Isolates from nasal swabs, pharyngeal swabs and stool samples were considered to be colonizers irrespective of the infection/colonization status of the patient. Therefore, patients were classified as infected or colonized, while isolates were classified as pathogens or colonizers. The isolates were identified as *P. aeruginosa* phenotypically and by PCR targeting two genes [18, 19].

Antimicrobial susceptibility and biofilm formation
Antimicrobial susceptibility was tested by the Kirby–Bauer disk diffusion method and the results were interpreted in accordance with CLSI guidelines [20]. Strains were categorized as multi-drug resistant (MDR) if they were non-susceptible to ≥3 antimicrobial classes, extensively drug resistant (XDR) if they were non-susceptible to all but ≤2 classes and pan-drug resistant (PDR) if they were non-susceptible to all tested antimicrobial agents.

Biofilm production was tested using the microtitre plate method [21], with some modification. Briefly, stationary-phase cultures of the strains in trypticase soy broth were diluted 1 : 100 and 100 μl was transferred to each of the four wells of a microtitre plate. The plates were incubated at 37°C in ambient air for 18–24 h. They were then washed and air dried. Adherent bacteria were fixed with methanol and stained with crystal violet. The adhering dye was dissolved in 30% acetic acid and the optical density was measured at 595 nm. *P. aeruginosa* strain PAO1 (Microbiological Type Culture Collection and Gene Bank, India) was used as a positive control.

Virulence factor PCRs and RAPD
Isolates were tested for the presence of the virulence factor genes *apr* (alkaline protease), *lasB* (elastase), *exoS*, *exoT*, *exoU* and *exoY* (type 3 secretion system effector enzyme genes), and *toxA* (exotoxin A) using PCR [22–24]. Each set of reactions was validated by an appropriate positive control – PAO1 for all genes except *exoU*, for which PA14 (obtained from Dr Lalitha Prajna, Aravind Eye Hospital, Madurai, India) was used.

The ERIC2 primer was used for genotyping by random amplification of polymorphic DNA (RAPD) [25]. The banding pattern obtained from the electrophoresis of PCR products was analysed using Gelcompar II (Applied-Maths, Kortrijk, Belgium) and UPGMA-generated dice coefficients were used to generate dendrograms.

Statistical analysis
The Chi-square independence test and the Mann–Whitney U test were used to identify statistically significant associations between host and organism factors. Binomial logistic regression was used to find the best set of factors associated with infection by *P. aeruginosa*. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software (IBM, Chicago, IL, USA).

RESULTS
A total of 547 patients were recruited into the study. The surveillance samples collected from these patients included 570 pharyngeal swabs, 582 nasal swabs, 475 urine samples and 288 stool samples. One hundred and ninety-six environmental samples from the hospital were also included. Of the in-patients included in this study, 11.2% (61/547) harboured *P. aeruginosa* as a pathogen and/or a colonizer.

Patient characteristics
Patient age ranged from 20 to 80 years. The length of hospital stay ranged from 2 to 52 days. Seventeen of the patients were admitted to the ICU for at least part of this duration. 62.3% (38/61) of the patients were culture-positive within 1 day of admission, with the mean time to culture positivity being 2.8 days (range 1–24 days). Four patients were transferred in from another hospital. When a foreign medical...
device (an endotracheal tube, or a urinary or central venous catheter) was present at the site from which *P. aeruginosa* was cultured, it was considered to be a relevant foreign device. Several patients suffered from co-morbidities such as diabetes mellitus (*n*=10), hypertension (*n*=20) and coronary artery disease (*n*=5). Three patients were classified as immunosuppressed (long-term steroid therapy; corticosteroids >10 mg day$^{-1}$ for >30 days). Four patients died while they were enrolled in the study.

Patients were classified into four categories:

1. infection by *P. aeruginosa* (*n*=32);
2. infection by multiple organisms (including *P. aeruginosa*) (*n*=12);
3. colonization by *P. aeruginosa*; no infection (*n*=12); and
4. colonization by *P. aeruginosa*; another infection present (*n*=5).

The other organisms isolated from patients with co-infection were *Acinetobacter* spp. (*n*=7), *Klebsiella pneumoniae* (*n*=5), *Citrobacter koseri* (*n*=2), *Enterobacter* spp. (*n*=2) and *Escherichia coli* (*n*=1). For the purpose of this classification, only bacterial infections were taken into account. Table 1 shows the distribution of host factors among infected and colonized patients. Among these, the duration of hospital stay correlated significantly with infection.

### Bacterial isolates

Seventy-six *P. aeruginosa* isolates were cultured from 61 patients, and 11 were cultured from environmental samples. Forty-six of the isolates cultured from patient samples were classified as pathogens, while the remaining 30 were classified as colonizers. The isolates included in the study were recovered from a number of sources, as shown in Table 2. The environmental isolates were primarily cultured from swabs taken from sinks and taps in the ward and the ICU, except two that were recovered from a bedside table and from an air sample in the ICU.

### Table 1. Host factors as a function of infection versus colonization status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Individuals with <em>P. aeruginosa</em> infection (<em>n</em>=44)</th>
<th>Individuals colonized with <em>P. aeruginosa</em> (<em>n</em>=12)</th>
<th>P value (Chi-square)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age&gt;50</td>
<td>33</td>
<td>8</td>
<td>0.064</td>
</tr>
<tr>
<td>Sex (M)</td>
<td>27</td>
<td>8</td>
<td>0.748</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7</td>
<td>3</td>
<td>0.433</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>3</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>Relevant foreign device</td>
<td>9</td>
<td>0</td>
<td>0.180</td>
</tr>
<tr>
<td>COPD</td>
<td>28</td>
<td>6</td>
<td>0.508</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>12</td>
<td>1</td>
<td>0.258</td>
</tr>
<tr>
<td>Previous TB</td>
<td>20</td>
<td>7</td>
<td>0.642</td>
</tr>
<tr>
<td>Hospital stay &gt;14d</td>
<td>9</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 2. Sources of *P. aeruginosa* isolates

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of isolates (<em>n</em>=87)</th>
<th>Pathogen (<em>n</em>=46)</th>
<th>Colonizer (<em>n</em>=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>39</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pharyngeal swab</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Urine</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Stool</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Wound Swab</td>
<td>1</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Environmental</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Virulence and antimicrobial susceptibility

The presence of virulence factors in pathogenic, colonizing and environmental strains is shown in Table 3. All strains were biofilm producers. Forty-nine isolates were both exoS- and exoT-positive, 11 were exoU- and exoT-positive and 3 were negative for all 3. None of the tested virulence factors were found to be significantly associated with infection. The presence of the exoU gene (*P*<0.003) caused infection by MDR strains (*P*<0.008) to correlate significantly with the duration of hospital stay >14 days. Length of hospital stay was considered to be a binary variable for this analysis (Chi-square test) and cut-offs of 7 and 14 days were analysed. For analysis as a continuous variable, the Shapiro–Wilk test for normality was employed, which showed that the data were not normally distributed. The Mann–Whitney U test found that both multi-drug resistance (*U*=232.5, *P*=0.009) and exoS and exoU positivity (*U*=130, *P*=0.003) were associated with longer hospital stays. These associations are depicted as box and whisker plots (Fig. S1, available with the online Supplementary Material). We found that positivity for exoS and exoU genes correlated strongly with multi-drug resistance (*P*<0.001). Further, exoU positivity correlated strongly with fluoroquinolone resistance (*P*<0.001). The environmental isolates were found to be significantly associated with exoS positivity (*P*<0.001).

The strains showed widely differing rates of susceptibility to various classes of antibiotics (Table 4). The highest resistance was seen against cefepime at 75.9 %, while none of the isolates were resistant to colistin. Multi-drug resistance was seen in 35 (40.2 %) of the isolates and 20 (23 %) were XDR. Antibiotic susceptibility was analysed as a function of the colonizer versus pathogen status of the isolates, as shown in Table 5. No statistically significant difference was found between the resistance rates (defined as the proportion of MDR strains) of the two classes. Nine (81.8 %) and 6 (54.5 %) of the 11 environmental isolates were MDR and XDR, respectively, as opposed to 34.2 and 18.4 % of the clinical isolates. This difference in the resistance profile is statistically significant (*P*<0.006).

### Random amplification of polymorphic DNA

A total of 85 isolates were typeable using the ERIC2 primer for RAPD. We obtained a good discriminatory index of 0.996
with this primer. Fig. 1 shows the dendrogram of the isolates and shows the percentage similarity between them. A high degree of genetic variability was observed among the 85 isolates, which resolved into 71 distinct RAPD patterns with >15% difference in UPGMA-generated dice coefficients.

The dendrogram in Fig. 2 shows the 30 colonizing isolates. There were two clones with two isolates that each showed a similarity of 85% or more. One was a susceptible clone isolated from urine from two separate patients and the other included XDR isolates, one each from urine and nasal swabs collected from different patients. The remaining 26 isolates were unique types.

When multiple samples from the same patient were analysed (Fig. 3) we found four clones that showed 85% similarity and were either colonizers or pathogens. These clones were identical in their resistance patterns and in the presence of virulence factors such as exoU, exoT and exoS.

**DISCUSSION**

We assessed a number of virulence factors, chosen for their association with infection in previous studies. All of our strains were biofilm producers. The other factors showed a more variable distribution, although most showed high prevalence. All isolates tested positive for at least two of the virulence factors. Eighty-four (96.6%) of our isolates were positive for at least one of the type 3 secretion system (T3SS) enzymes. As expected, exoU had the lowest prevalence at 27.6% [26, 27]. We also found that exoU positivity correlated strongly with levofloxacin resistance (P<0.001), as previously reported [27]. Mutations in the gyrA gene (responsible for fluoroquinolone resistance) have been associated with the presence of the T3SS genes [28]. It has been suggested that co-selection of exoU and fluoroquinolone resistance is occurring, particularly in fluoroquinolone-rich environments [29]. However, other workers have failed to find such a correlation [26]. We found that the presence of the genes exoS and exoU was mutually exclusive.

exoU and exoS positivity also correlated strongly with the presence of multi-drug resistance (P<0.001). MDR strains of *P. aeruginosa* have been reported to have a high incidence of the exoU gene [30]. The persistence of MDR strains in the host may allow for the development of virulent genotypes [31, 32]. We also found environmental isolates to be significantly more likely to be exoU-positive than clinical isolates (P=0.001), which is in accordance with previous findings [23]. Another study found that environmental isolates from oil contaminated soils produced virulence factors, including pscJ, a component of the type 3 secretion system needle complex [33]. Other studies have also noted the presence of virulence factors in environmental strains, although at lower rates than clinical isolates [22, 26]. Isolation of exoU-positive strains, which are the most virulent

### Table 3. Virulence factors as a function of colonization/infection status

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Pathogens [n=46 (%)]</th>
<th>Colonizers [n=30(%)]</th>
<th>Environmental [n=11(%)]</th>
<th>P value (P versus C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>apr</td>
<td>37 (80.4)</td>
<td>27 (90)</td>
<td>8 (72.7)</td>
<td>0.671</td>
</tr>
<tr>
<td>lasB</td>
<td>37 (80.4)</td>
<td>24 (80)</td>
<td>9 (81.8)</td>
<td>1.000</td>
</tr>
<tr>
<td>exoS</td>
<td>35 (76)</td>
<td>22 (73.3)</td>
<td>2 (18.2)</td>
<td>1.000</td>
</tr>
<tr>
<td>exoT</td>
<td>36 (78.2)</td>
<td>23 (76.7)</td>
<td>5 (45.4)</td>
<td>0.424</td>
</tr>
<tr>
<td>exoU</td>
<td>8 (17.4)</td>
<td>8 (26.7)</td>
<td>8 (72.7)</td>
<td>0.666</td>
</tr>
<tr>
<td>exoY</td>
<td>42 (91.3)</td>
<td>29 (96.7)</td>
<td>11 (100)</td>
<td>0.567</td>
</tr>
<tr>
<td>luxA</td>
<td>40 (87)</td>
<td>25 (83.3)</td>
<td>11 (100)</td>
<td>0.348</td>
</tr>
<tr>
<td>Biofilm</td>
<td>46 (100)</td>
<td>30 (100)</td>
<td>11 (100)</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 4. Antibiotic susceptibility of *P. aeruginosa* isolates (n=87)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible (%)</th>
<th>Non-susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime</td>
<td>54 (62)</td>
<td>33 (37.9)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>21 (24.1)</td>
<td>66 (75.9)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>57 (65.3)</td>
<td>30 (34.5)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>52 (59.8)</td>
<td>35 (40.2)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>58 (66.7)</td>
<td>29 (33.3)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>64 (73.6)</td>
<td>23 (26.4)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>61 (70.1)</td>
<td>26 (29.9)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>46 (52.9)</td>
<td>41 (47.1)</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>59 (67.8)</td>
<td>28 (32.2)</td>
</tr>
<tr>
<td>Colistin</td>
<td>87 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

### Table 5. Antibiotic susceptibility as a function of colonization/infection status

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Pathogens [n=46 (%)]</th>
<th>Colonizers [n=30 (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime</td>
<td>16 (34.8)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>32 (69.6)</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>9 (19.6)</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>16 (34.8)</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>10 (21.7)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8 (17.4)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>9 (19.6)</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>18 (39.1)</td>
<td>15 (50)</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>10 (21.7)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>Colistin</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Aditi et al., Journal of Medical Microbiology 2017;66:1489–1498

Fig. 1. UPGMA clustering dendrogram indicating the percentage similarities between the RAPD pattern of the *P. aeruginosa* isolates (n=85). S, sensitive; MDR, multi-drug resistant; XDR, extensively drug resistant; ET, endotracheal aspirate; WS, wound swab; Th, throat/pharyngeal; Reg. no, patient registration number.
among the T3SS-positive strains of *P. aeruginosa*, was significantly associated with length of hospital stay (*P*=0.003). This may be indicative of the severity of disease; however, no significant association with mortality was seen. Persistence in the host was not assessed in our study.

None of these virulence factors were found to be significantly associated with the presence of infection. Although T3SS is a major virulence factor, T3SS-negative strains are also known to be pathogenic [34]. In patients with ventilator-associated pneumonia, infection by T3SS-positive strains has been shown to be associated with severe disease as well as increased risk of persistence and relapse [35, 36]. Our results are in concordance with a recent study that also found no significant association in patients with respiratory tract infections [14]. Another study found no significant relationship between cytotoxicity and infection [37]. A recent study reported extensive similarities between infection- and colonization-associated *Neisseria meningitidis*, indicating that the same strains cause infection and colonization [38]. Therefore, it seems likely that the strains that have the ability to colonize the host may also cause infection.
During colonization as well as infection, the most virulent pathogens are those that are best able to compete in new niches. Resistant and/or virulent strains are more likely to replace the susceptible commensals, particularly in a nosocomial environment with high antibiotic pressure. Resistance to antibiotics may be considered to be a virulence-like factor [39]; just as antimicrobial resistance among commensals has increased globally [40], virulence may follow the same trend. We found that colonizing strains were more resistant to most antibiotics, though the difference was not statistically significant. This is despite the fact that 66% (20/30) of the colonizing isolates were isolated within 48 h of admission.

Environmental isolates were found to be more resistant, which has been reported previously [41]. Nine (81.8%) and 6 (54.5%) of the 11 environmental isolates were MDR and XDR, respectively, as opposed to 34.2 and 18.4% of the clinical isolates (P=0.006). P. aeruginosa adapts well to the nosocomial environment and acquires resistance rapidly. This is the reason that the relative prevalence of P. aeruginosa in hospital settings is increasing faster than that of other
organisms that acquire resistance at a slower rate, such as *E. coli* [39]. In our study, we found that sinks in the ICU and ward were niches for XDR *P. aeruginosa*.

Our study has some important limitations. One limitation is that we assessed the presence of virulence factor genes or measured virulence phenotypes *in vitro*. Thus, we only assessed the potential for an isolate to express a given phenotype in the patient, not whether they were actually expressed in the host. Further, patients were only followed clinically for the duration of their stay in the hospital, and subsequent hospital admissions and their outcomes were disregarded.

Eighty-five isolates were typeable by RAPD. One clone contained two identical isolates, one a nasal colonizer and the other from an air sample in the ICU to which the patient was admitted during the same time period. An XDR clone (>85% similarity) comprised environmental isolates from the sinks and taps of the ward and the ICU. This strain has colonized these spatially dissociated sites, probably through the hands of healthcare personnel and represents a potential source of infection in the hospital environment. Three clones (>85% similarity) contained paired isolates of colonizers and pathogens from the same patient. For all three of these patients, the colonizing isolate was cultured from samples collected on the day of admission, while the pathogenic

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**Fig. 3.** UPGMA clustering dendrogram indicating the percentage similarities between the RAPD pattern of *P. aeruginosa* isolates from multiple samples from patients (*n*=27). S, Sensitive; MDR, multi-drug resistant; XDR, extensively-drug resistant; ET, endotracheal aspirate; WS, wound swab; Th, throat/pharyngeal; Reg. no, patient registration number.
isolate was cultured on the first, fourth and fifth days after admission, which may indicate progression to infection. We found no evidence of an outbreak or of transmission between patients.

In summary, the presence of virulence factors genes was not found to be significantly associated with infection in our study. Although more virulent strains were found to be more resistant, we did not find any significant association between resistance to antimicrobials and the presence of infection.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study was ethically approved by the Institutional Ethics committee, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi and written informed consent was taken from the patients.

References


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