Identification of carbapenem-resistant *Pseudomonas aeruginosa* in selected hospitals of the Gulf Cooperation Council States: dominance of high-risk clones in the region

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**Abstract**

**Purpose.** The molecular epidemiology and resistance mechanisms of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) were determined in hospitals in the countries of the Gulf Cooperation Council (GCC), namely, Saudi Arabia, the United Arab Emirates, Oman, Qatar, Bahrain and Kuwait.

**Methodology.** Isolates were screened for common carbapenem-resistance genes by PCR. Relatedness between isolates was assessed using previously described genotyping methods: an informative-single nucleotide polymorphism MassARRAY iPLEX assay (iPLEX20SNP) and the enterobacterial repetitive intergenic consensus (ERIC)-PCR assay, with selected isolates being subjected to multilocus sequence typing (MLST). Ninety-five non-repetitive isolates that were found to be resistant to carbapenems were subjected to further investigation.

**Results/Key findings.** The most prevalent carbapenemase-encoding gene, *bla*VIM-type, was found in 37/95 (39 %) isolates, while only 1 isolate (from UAE) was found to have *bla*IMP-type. None of the CRPA were found to have *bla*OXY-type or *bla*KPC-type.

We found a total of 14 sequence type (ST) clusters, with 4 of these clusters being observed in more than 1 country. Several clusters belonged to the previously recognized internationally disseminated high-risk clones ST357, ST235, ST111, ST233 and ST654. We also found the less predominant ST316, ST308 and ST823 clones, and novel MLST types (ST2010, ST2011, ST2012 and ST2013), in our collection.

**Conclusion.** Overall our data show that ‘high-risk’ CRPA clones are now detected in the region and highlight the need for strategies to limit further spread of such organisms, including enhanced surveillance, infection control precautions and further promotion of antibiotic stewardship programmes.
INTRODUCTION

Pseudomonas aeruginosa is a common opportunistic pathogen that is associated with hospital-acquired infections (HAIs). Multicentre surveys from the USA have shown that P. aeruginosa comprises between 14 and 21% of all pathogens causing ventilator-associated pneumonia (VAP) [1–3]. The rate of P. aeruginosa association with VAP in Asia can be as high as 42% [4]. In Saudi Arabia, a prospective cohort of VAP from an adult general intensive care unit (ICU) found that 21.7% were caused by P. aeruginosa [5].

The success of this pathogen is partially due to the multidrug-resistant (MDR) phenotype that P. aeruginosa demonstrates [6], which more recently has been attributed to the international spread of certain successful clones [7–10]. In the Middle East, and the countries of the Cooperation Council for the Arab States of the Gulf (GCC; Saudi Arabia, the United Arab Emirates (UAE), Oman, Kuwait, Qatar and Bahrain) in particular, the prevalence of carbapenem-resistant P. aeruginosa (CRPA) has increased dramatically over the last decade [11]. Al Johani et al. carried out a retrospective analysis of Gram-negative bacilli isolated from the adult ICU of King Abdulaziz Medical City in Riyadh, Saudi Arabia. They found a significant decline in the susceptibility of P. aeruginosa to carbapenem, from 66% in 2004 to 26% in 2009 [12]. CRPA are often resistant to other classes of antibiotics [6], which can impede effective treatment, leading to increased morbidity and mortality worldwide [6, 13].

The mechanisms contributing to carbapenem resistance in P. aeruginosa can include alteration of the OprD outer membrane porin, multidrug efflux pumps [6], as well as mobile genetic elements such as plasmid-encoded carbapenemases. The most notable of these are the plasmid-mediated metallo-β-lactamases (MBLs), which are widespread and have been reported around the world [7, 9]. Previous reports on CRPA isolates from the GCC states show that carbapenem resistance is most often associated with VIM-type and IMP-type MBL enzymes [11, 14, 15]. However, other carbapenemase encoding genes such as blaNDM-type [10, 16–21] and blaKPC-type [22–28] have been increasingly reported in P. aeruginosa isolated from elsewhere around the world.

Several reports have highlighted the international spread of successful 'high-risk clones' of P. aeruginosa. Using multilocus sequence typing (MLST), it has been established that P. aeruginosa belonging to sequence types 111, 235 and 175 are the most notable high-risk clones. These clones are associated with an MDR phenotype, including MBL producers [7, 9, 10, 29]. The GCC region witnesses heavy travel activity as a result of the high influx of an international workforce [30] and Hajj [31]. Since travel is known to be a risk factor for spreading MDR bacteria [32], this may facilitate the dissemination of MDR high-risk P. aeruginosa clones into this region.

In our initial investigations we observed and described an extended-spectrum beta-lactamase (ESBL) PME-1-producing P. aeruginosa in Qatar [33]. In this study, we aimed to determine the broad molecular characteristics of CRPA in the GCC states. To the best of our knowledge, this is the first region-wide snapshot analysis of the molecular epidemiology of CRPA in hospitals within the GCC.

METHODS

Bacterial isolates

Between July 2011 and January 2013, P. aeruginosa were collected from seven participating institutes across the GCC states [two hospitals in Saudi Arabia, and one hospital each from the UAE, Kuwait, Qatar, Oman and Bahrain] (Table 1). No CRPA were contributed by our study hospital in Kuwait. These hospitals are part of a region-wide collaborative study on MDR Gram-negative bacilli [33–35]. P. aeruginosa were identified and tested for their susceptibility to a panel of antimicrobials using a semi-automated MIC-based systems in each clinical microbiology laboratory. Isolates were included on the basis of showing a non-susceptible phenotype to imipenem (MIC ≥8 µg ml⁻¹), and/or meropenem (MIC ≥8 µg ml⁻¹) using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [36]. Only one isolate per patient was included, and the isolates originated from a range of clinical specimens. All carbapenem-non-susceptible isolates were sent to the research laboratory at the University of Queensland Centre for Clinical Research (UQCCR), Australia for further investigation.

Antibiotic susceptibility testing

All non-susceptible isolates referred to the UQCCR underwent confirmatory disk diffusion susceptibility testing following the methodology and breakpoints defined by the EUCAST [36] for the following antimicrobial agents: ceftazidime, cefepime, pipercillin/tazobactam, imipenem, meropenem, aztreonam, gentamicin, amikacin and ciprofloxacin.

PCR for carbapenemase genes and sequencing

Genomic DNA was extracted using the UltraClean Microbial DNA Isolation kit (Qiagen, Germany) as recommended by the manufacturer. Following confirmation of species identification by PAduplex PCR [37], each isolate was screened using blaVIM-type PCR, blaIMP-subtype PCR and blaNDM-type–blaKPC-type duplex PCR using previously described primer sets [34, 35, 38]. All PCR reactions were carried out using GoTaq Green Master Mix (Promega, USA). The resulted PCR products for blaVIM-type and blaIMP-subtype were sequenced to identify the variants.

Genotyping of carbapenem-resistant P. aeruginosa

Each CRPA isolate was subjected to two independent P. aeruginosa genotyping methods comprising an iPLEX MassARRAY single nucleotide polymorphism method (iPLEX20SNP) and an enterobacterial repetitive intergenic consensus (ERIC) PCR assay. Representative isolates from each combined iPLEX20SNP-ERIC PCR cluster, along with four randomly selected singleton isolates, were selected for MLST.
Table 1. Summary of CRPA clinical isolates in the GCC states

<table>
<thead>
<tr>
<th>Location</th>
<th>Hospital</th>
<th>Category</th>
<th>Bed size</th>
<th>Semi-automated system used for species identification and antibiotic sensitivity*</th>
<th>No. of carbapenem resistant P. aeruginosa</th>
<th>No. (%) of carbapenem resistance mechanisms†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riyadh, Saudi Arabia</td>
<td>King Abdulaziz Medical City</td>
<td>Tertiary and academic</td>
<td>1000</td>
<td>Vitek II</td>
<td>43 (30)</td>
<td>VIM type</td>
</tr>
<tr>
<td>Khobar, Saudi Arabia</td>
<td>King Fahad University Hospital</td>
<td>Tertiary and academic</td>
<td>450</td>
<td>Vitek II</td>
<td>19 (37)</td>
<td>IMP type</td>
</tr>
<tr>
<td>Abu Dhabi, United Arab Emirates</td>
<td>Sheikh Zayed Military hospital</td>
<td>Tertiary</td>
<td>365</td>
<td>Vitek II</td>
<td>18 (50)</td>
<td>IMP type</td>
</tr>
<tr>
<td>Muscat, Oman</td>
<td>The Royal Hospital</td>
<td>Teaching and tertiary</td>
<td>750</td>
<td>Phoenix</td>
<td>1 (0)</td>
<td>VIM type</td>
</tr>
<tr>
<td>Doha, Qatar</td>
<td>Hamad Medical Cooperation Complex</td>
<td>Tertiary and teaching</td>
<td>&gt;1300</td>
<td>Phoenix</td>
<td>7 (43)</td>
<td>IMP type</td>
</tr>
<tr>
<td>Manama, Bahrain</td>
<td>Samlanaya Medical Complex</td>
<td>Tertiary and teaching</td>
<td>1000</td>
<td>Phoenix</td>
<td>7 (71)</td>
<td>IMP type</td>
</tr>
<tr>
<td>Total no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95 (39)</td>
<td>VIM type</td>
</tr>
</tbody>
</table>

*Vitek II, bioMérieux; Phoenix, Becton-Dickinson.
†All isolates were negative for blanDM-type or blarKPC-type PCR.

iPLEX20SNP MassARRAY genotyping

The iPLEX20SNP assay was performed as previously described [39]. Briefly, the method targets 20 informative SNPs arising from 7 *P. aeruginosa* housekeeping genes (acsA, aroE, guaA, mutL, nuoD, ppsA and trpE), which are distinguished using the Sequenom MassARRAY iPLEX platform and used to predict an MLST type. Using these data, the sequence types (STs) were determined by comparing the isolate profiles with the 1198 distinct 20 SNP profiles generated from in silico analysis of the 1982 concatenated sequences downloaded from the P. aeruginosa MLST database (http://pubmlst.org/paeruginosa/; accessed 20 April 2015) as previously described [39].

ERIC-PCR

ERIC-PCR analysis was performed as described previously [40]. In brief, 100 ng of extracted DNA was amplified using primers targeting ERIC sequences and thereafter subjected to electrophoresis (80 V) in 1.2 % agarose for 180 min. ERIC-PCR banding patterns were compared using FPQuest cluster analysis software, version 4.5 (Bio-Rad Laboratories Pty Ltd, USA). Isolates producing fingerprints related by arithmetic mean (UPGMA) were allocated to the same ERIC-PCR type. Clusters were defined based on >90 % ERIC-PCR banding pattern similarity, as well as the predicted MLST profile based on the iPLEX20SNP assay.

MLST

A total of 19 isolates, selected to represent each cluster (n=15) and some singletons (n=4), from the six countries were also subjected to sequencing-based MLST to confirm the precise ST. These isolates comprised one isolate each from clusters A, B, C, D, E, F, G, H, I, J, K, M and N, two isolates from the large cluster L (Table 2) and four isolates that were identified as singletons (Table 3). The iPLEX20SNP assay typically does not distinguish all individual STs, but rather places most iPLEX20SNP types into groups of closely related STs [39]. Paired-end libraries of whole genomic DNA of the isolates were prepared via the Nextera XT DNA Sample Preparation kit and sequenced by the Illumina HiSeq platform (Illumina, USA). The 100 bp pair-end reads were de novo assembled using the CLC Genomic Workbench with a minimum contig length of 200 bp. One hundred and sixty-seven contigs were assembled with a depth coverage of ca. 100 ×. The STs were determined using in silico MLST analysis (https://cge.cbs.dtu.dk/services/MLST/) [41].

RESULTS

Bacterial isolates and carbapenem susceptibility

A total of 95 non-repetitive isolates that were non-susceptible to imipenem and/or meropenem were referred for further susceptibility testing. The numbers of CRPA in each participating hospital were as follows: Saudi Arabia – Riyadh, 43; Saudi Arabia – Khobar, 19; United Arab of Emirates, 18; Oman, 1; Qatar, 7; Bahrain, 7 (Table 1). In total, 90/95 isolates (95 %) showed resistance to both imipenem and meropenem, whereas 5 isolates were only resistant to 1 of these agents, confirming the semi-automated MIC test results provided by the clinical laboratories. Two isolates from Saudi Arabia – Khobar were resistant to imipenem, but sensitive to meropenem. Three isolates (one from Saudi Arabia – Khobar and two from Saudi Arabia – Riyadh) were resistant to meropenem, but showed a sensitive phenotype to imipenem based on the EUCAST criteria. Carbapenem co-resistance for the 95 CRPA isolates was found to be as follows: ceftazidime (60 %), aztreonam (39 %), gentamicin (62 %), amikacin (50 %), ciprofloxacin (80 %) and piperacillin/tazobactam (43 %).

Carbapenemase-encoding genes

Thirty-eight of the 95 (40 %) CRPA isolates showed a positive result for carbapenem-encoding gene PCRs. The carbapenem-encoding gene blanDM-type was encountered most frequently (n=37/95 (39 %)) and only one isolate from the UAE was found to be carrying the blarKPC-subtype 1 gene. No isolates were positive for blanNDM-type or blarKPC-type. Table 1 shows the geographical distribution of the VIM-
type MBL-producing isolates, with 78 % (N=29) arising from Saudi Arabia and the UAE.

**Genotyping and clonality**

**iPLEX20SNP analysis**

Complete SNP profiles were obtained for 91/95 (95.8 %) isolates by the iPLEX20SNP assay. Twenty-eight different MLST profiles were predicted for them. The three most common predicted MLST profile grouping were groups P-15 (n=23 isolates), P-01 (n=15 isolates) and P-05 (n=13 isolates). The other predicted MLST profile grouping were less abundant, with some profiles only occurring once (Table 3). The assay also predicted isolates with possible novel STs (n=5). Four isolates had incomplete profiles, as one or more SNPs could not be characterized by the iPLEX20SNP assay, which was likely caused by sequence variation in the primer targets [39].

**ERIC-PCR analysis**

ERIC-PCR analysis combined with predicted MLST profiles identified a total of 14 clusters (clusters A to N, comprising 52 isolates; see Table 2 and Fig. S1, available in the online version of this article) and 43 singletons among the 95 study isolates (Table 3). The largest cluster (L) included 17/95 isolates (18 %) comprising isolates from the two hospitals in Saudi Arabia and Bahrain. Of interest, all cluster L isolates had the same profile, P-15. Cluster A consisted of five Saudi isolates (four from Riyadh and one from Khobar). Two clusters (B and K) each consisted of four isolates, while clusters G and M had three isolates each. The remaining eight clusters included two isolates each (Table 2; see also Fig. S1).

**Table 2. Clustering results based on the ERIC-PCR banding patterns (>90 % similarity) and predicted MLST profiles of the 52 carbapenem-resistant Pseudomonas aeruginosa isolates from the GCC states**

<table>
<thead>
<tr>
<th>Cluster†</th>
<th>Predicted MLST profile</th>
<th>Predicted MLST profile grouping</th>
<th>No. of isolates</th>
<th>Carbapenemase genes detected</th>
<th>Sequence type of the isolates selected for MLST‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>357, 746, 1153, 1424, 1679</td>
<td>P-1</td>
<td>5</td>
<td>ND§</td>
<td>ST357</td>
</tr>
<tr>
<td>B</td>
<td>357, 746, 1153, 1424, 1679</td>
<td>P-1</td>
<td>4</td>
<td>ND</td>
<td>ST357</td>
</tr>
<tr>
<td>C</td>
<td>357, 746, 1153, 1424, 1679</td>
<td>P-1</td>
<td>2</td>
<td>ND</td>
<td>ST357</td>
</tr>
<tr>
<td>D</td>
<td>227, 230, 235, 533, 534, 696, 745, 976, 989, 1457, 1592, 1788, 1789, 1829</td>
<td>P-5</td>
<td>2</td>
<td>ND</td>
<td>ST357</td>
</tr>
<tr>
<td>E</td>
<td>111, 113, 284, 584, 600, 772, 1314, 1319, 1782, 1783, 1791</td>
<td>P-9</td>
<td>2</td>
<td>blavIM-2 or 11</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>227, 230, 235, 533, 534, 696, 745, 976, 989, 1457, 1592, 1788, 1829</td>
<td>P-5</td>
<td>2</td>
<td>blavIM-24</td>
<td>ST235</td>
</tr>
<tr>
<td>G</td>
<td>227, 230, 235, 533, 534, 696, 745, 976, 989, 1457, 1592, 1788, 1789, 1829</td>
<td>P-5</td>
<td>3</td>
<td>blavIM-2, 17 or 48</td>
<td>ST235</td>
</tr>
<tr>
<td>H</td>
<td>227, 230, 235, 533, 534, 696, 745, 976, 989, 1457, 1592, 1788, 1829</td>
<td>P-5</td>
<td>2</td>
<td>blavIM-2, 17 or 48</td>
<td>ST235</td>
</tr>
<tr>
<td>I</td>
<td>645, 964, 1696</td>
<td>P-17</td>
<td>2</td>
<td>blavIM-2, 17 or 48</td>
<td>ST654</td>
</tr>
<tr>
<td>J</td>
<td>89, 307, 308, 662, 1028, 1410, 1488, 1746, 1794</td>
<td>P-18</td>
<td>2</td>
<td>blavIM-2, 17 or 48</td>
<td>ST308</td>
</tr>
<tr>
<td>K</td>
<td>658, 823, 1017, 1142, 1676, 1813</td>
<td>P-25</td>
<td>4</td>
<td>blavIM-2</td>
<td>ST823</td>
</tr>
<tr>
<td>L</td>
<td>233, 375, 1446, 1699, 1701, 1702, 1712, 1760</td>
<td>P-15</td>
<td>17</td>
<td>blavIM-2, 17 or 48</td>
<td>ST233</td>
</tr>
<tr>
<td>M</td>
<td>233, 375, 1446, 1699, 1701, 1702, 1712, 1760</td>
<td>P-15</td>
<td>3</td>
<td>blavIM-2</td>
<td>ST233</td>
</tr>
<tr>
<td>N</td>
<td>NT4</td>
<td>P-29</td>
<td>2</td>
<td>blavIM-2</td>
<td>Novel ST2013</td>
</tr>
</tbody>
</table>

*For a full dendrogram, see Fig. S1.
†Clusters are defined based on >90 % ERIC-PCR banding pattern similarity as well as the predicted MLST profile based on the iPLEX20SNP assay.
‡One isolate from each combined iPLEX20SNP ERIC-PCR cluster was selected for MLST.
§ND, not detected.
||Not fully determined variant, due to repeated technical difficulties.
Well-defined clusters by location were observed in clusters C, D, G, I, J, M and N (Table 2), whereas the remaining clusters included isolates from two or more locations. Isolates harbouring \textit{bla\textsubscript{VIM}}-type were scattered throughout the CRPA collection (Tables 2 and 3). All isolates in clusters F, K, L, and M were \textit{bla\textsubscript{VIM}}-type producers (Table 2).

**MLST**

Using MLST, we identified eight different STs (i.e. ST111, ST233, ST235, ST308, ST316, ST357, ST654 and ST823) and four novel STs (i.e. ST2010, ST2011, ST2012 and ST2013); all were assigned to the 19 representative isolates. All STs identified via \textit{in silico} whole-genome sequence analysis matched with one of the predicted STs identified by the iPLEX20SNP assay. Selected isolates representing clusters A, B and C were found to be ST357, suggesting that all isolates \((n=11)\) under these clusters may belong to ST357. Isolates belonging to cluster D, which consisted of two isolates, were found to be novel sequence type ST2010. Cluster E \((N=2)\) had an ST111 isolate. Selected isolates representing clusters F, G and H suggested that a total of 12 isolates belonged to ST235. Selected isolates representing clusters I and J, which had two isolates each, were found to be ST654 and ST308, respectively. The isolates selected to represent cluster K \((N=4)\) were found to be ST823. Two isolates were selected to represent cluster L, which consisted of 17 isolates. They were found to be ST233. An ST233 isolate was also found in

### Table 3. Singleton profiles of 43 carbapenem-resistant \textit{P. aeruginosa} isolates from the GCC states*

<table>
<thead>
<tr>
<th>Location, no. of isolates</th>
<th>Predicted MLST profile</th>
<th>Predicted MLST profile grouping</th>
<th>No. of isolates</th>
<th>Carbapenemases</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saudi Arabia – Riyadh, 20</td>
<td>830, 1076, 1112</td>
<td>P-2</td>
<td>3</td>
<td>ND†</td>
<td>NP‡</td>
</tr>
<tr>
<td></td>
<td>357, 746, 1153, 1424, 1679, 1982</td>
<td>P-1</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>233, 375, 1446, 1699, 1700, 1702, 1712, 1760</td>
<td>P-15</td>
<td>3</td>
<td>\textit{bla\textsubscript{VIM}}, \textit{17 or 48}</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>1091</td>
<td>P-16</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>612</td>
<td>P-19</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>89, 307, 308, 662, 1028, 1410, 1488, 1746, 1794</td>
<td>P-18</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>NT1</td>
<td>P-21</td>
<td>1</td>
<td>ND</td>
<td>ST2012§</td>
</tr>
<tr>
<td></td>
<td>NT2</td>
<td>P-20</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>564, 604, 1188</td>
<td>P-22</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>1211</td>
<td>P-23</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>463</td>
<td>P-27</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>65, 107, 109, 253, 297, 338, 342, 377, 532, 773, 815, 923, 1110, 1363, 1570, 1607, 1619</td>
<td>P-28</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>Incomplete profile</td>
<td>P-30</td>
<td>4</td>
<td>\textit{bla\textsubscript{VIM}}, \textit{(n=1)}</td>
<td>NP</td>
</tr>
<tr>
<td>Saudi Arabia – Khobar, 7</td>
<td>357, 746, 1153, 1424, 1679, 1982</td>
<td>P-1</td>
<td>3</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>P-11</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>639, 853</td>
<td>P-13</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>865</td>
<td>P-16</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td>UAE, 13</td>
<td>830, 1076, 1112</td>
<td>P-2</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
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<tr>
<td></td>
<td>227, 230, 235, 533, 534, 696, 745, 976, 989, 1457, 1592, 1788, 1789, 1829</td>
<td>P-5</td>
<td>4</td>
<td>\textit{bla\textsubscript{IMP}}, \textit{(n=1)}, \textit{bla\textsubscript{VIM}}, \textit{(n=3)}</td>
<td>ST235</td>
</tr>
<tr>
<td></td>
<td>61, 223, 309, 311, 316, 325, 361, 383, 458, 1251, 1310, 1803</td>
<td>P-6</td>
<td>2</td>
<td>ND</td>
<td>ST316§</td>
</tr>
<tr>
<td></td>
<td>664, 1167, 1337</td>
<td>P-8</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>NT3</td>
<td>P-12</td>
<td>1</td>
<td>ND</td>
<td>ST2011</td>
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<td>89, 307, 308, 662, 1028, 1410, 1488, 1746, 1794</td>
<td>P-18</td>
<td>2</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>170, 367, 373, 997, 1315, 1697</td>
<td>P-24</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>654, 964, 1696</td>
<td>P-17</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>1575, 1756</td>
<td>P-31</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td>Qatar, 3</td>
<td>882, 1151, 1233</td>
<td>P-7</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>159, 175, 619</td>
<td>P-10</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>631, 1019, 1284, 1486, 1567</td>
<td>P-14</td>
<td>1</td>
<td>ND</td>
<td>NP</td>
</tr>
</tbody>
</table>

*For a full dendrogram, see Fig. S1.
†ND, not detected.
‡NP, not performed.
§The ST of one isolate was characterized.
||Novel STs.
cluster M, which consisted of three isolates. A novel ST2013 was identified in cluster N (Table 2 and Fig. S1).

**DISCUSSION**

Here we describe the molecular characterization of clinical CRPA isolated from selected hospitals in the GCC States. We found that VIM type was the most commonly identified carbapenemase in our CRPA collection. This finding reflects earlier reports from Saudi Arabia [11, 14, 15] and neighbouring Egypt [16, 42–44]. To the best of our knowledge, our findings are the first to identify VIM-producing *P. aeruginosa* in the UAE, Bahrain and Qatar.

Another commonly found MBL in *P. aeruginosa* is the IMP type [38, 45]. However, in our collection we only found one CRPA carrying blaIMP-1 and it is the first to be reported from the UAE. The rarity of IMP type-producing *P. aeruginosa* in the region is consistent with previous investigations [11]. Memish *et al.* conducted a national survey of Gram-negative bacteria in Saudi Arabia, and none of the 40 *P. aeruginosa* isolated from seven Saudi cities produced an IMP-type MBL [14]. However, Al-Agamy *et al.* only identified only one IMP-7-producing *P. aeruginosa* out of 35 CRPA collected at a tertiary hospital in Riyadh, Saudi Arabia [46].

Despite the emergence and international spread of *P. aeruginosa* harbouring blakPC-type and bladSM-type [10, 16, 26], we did not encounter any. Similarly, the survey of MDR *P. aeruginosa* in Saudi Arabia did not find these enzymes [14]. NDM-producing *P. aeruginosa* have been reported from nearby Egypt [16, 43], and among the internationally disseminated high-risk clone ST233 [16], which was also found among our collection. We found that approximately 60 % of our CRPA collection were negative for common carbapenemase genes. This complements the findings of Memish *et al.*, as 72 % of their Saudi-collected isolates were also negative. Carbapenem resistance in *P. aeruginosa* can arise through various other mechanisms, including loss of the carbapenem-specific outer-membrane porin OprD, the overexpression of efflux pumps [6], or other carbapenemase enzymes, which we did not test for.

Overall, we found that multiple clusters of clonal CRPA isolates exist in different hospitals across the Gulf region. The most notable is the L cluster, which consists of 17 VIM-2 and VIM-2, 17 or 48 CRPA isolates from Saudi Arabia and Bahrain, and belongs to the international high-risk clone ST233. Isolates belonging to this cluster – and others – demonstrate very similar resistance phenotypes to multiple antibiotics. We also noted that the majority of our CRPA collection belonged to other well-known and internationally disseminated high-risk clones, such as ST111, ST235, ST357 and ST654. *P. aeruginosa* belonging to these STs are disseminated in the UK and are associated with MBL production [10]. CRPA isolated from Colombia were also found to be associated with ST111 and ST235, and to carry blavIM-type and blakPC-type, respectively [7]. Our CRPA isolates of interest included the previously described PME-1 ESBL, which also belongs to ST654 [33]. These findings are worrying, because the existence of high-risk clones in the GCC might facilitate further dissemination of highly resistant *P. aeruginosa* in local hospitals.

In summary, we analysed clinical CRPA isolates obtained from selected hospitals across the GCC states. VIM-type MBL was the most common carbapenemase. We identified clusters belonging to internationally disseminated high-risk clones. Knowing the dissemination level of highly resistant CRPA in the region’s hospitals may stimulate the enhancement of infection control precautions and the optimization of antibiotic stewardship in hospitals, community pharmacies and within agricultural settings. The limitations of our study include the fact that only a small number of hospitals contributed isolates – therefore, this is not necessarily a representation of the status of CRPA in the region as a whole. It was also not possible to keep track of the denominator, and so it was not possible to determine the prevalence of CRPA. Not all of the isolates were subjected to sequencing-based MLST, some rather had their MLST types inferred by less discriminatory SNP-based profiling. Last, but not least, the intrinsic resistance mechanisms and others carbapenemase enzymes were not tested in this study, and the specific VIM and IMP types were not fully ascertained. The latter could have been resolved if we had subjected all isolates to WGS, but when this study was conducted WGS was not affordable at our institute.

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**Conflicts of interest**

D. L. P. has received honoraria for advisory board participation from AstraZeneca, Bayer, Cubist, Pfizer and Merck, not relating to this work. D. M. W. reports grants from SpeeDx Pty Ltd unrelated to the submitted work. All other authors confirmed that they have no conflicts of interest to declare.

**Ethical statement**

This project (number 2011000674) was approved by the Human Ethics Committee at The University of Queensland. It was agreed that collaborators from each of the participating hospitals in the GCC states had to
obtain Institutional Review Board (IRB) approvals prior to participation. Advice and assistance from the coordinating team at UQCCR was provided to some institutes. The Ministry of National Guard, Health Affairs, and King Abdullah International Medical Research Centre in Saudi Arabia approved the participation of King Abdulaziz Medical City in Riyadh in this study and supported project ref. MRBC/193/12.

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