JOURNAL OF CLINICAL MICROBIOLOGY, July 2010, p. 2563–2564 Vol. 48, No. 7
0095-1137/10/$12.00 doi:10.1128/JCM.01905-09

Multilocus Sequence Types of Carbapenem-Resistant *Pseudomonas aeruginosa* in Singapore Carrying Metallo-β-Lactamase Genes, Including the Novel bla_{IMP-26} Gene[▽]

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Received 26 September 2009/Returned for modification 13 October 2009/Accepted 26 April 2010

Nine imipenem-resistant *Pseudomonas aeruginosa* isolates were found to contain a variety of metallo-β-lactamase genes, including bla_{IMP-1}, bla_{IMP-7}, bla_{VIM-2}, bla_{VIM-6}, and the novel bla_{IMP-26}. Multilocus sequence typing showed a diversity of sequence types. Comparison with isolates from an earlier study showed that the epidemic clones from 2000 have not become established.

Carbapenem-resistant *Pseudomonas aeruginosa* is an increasing problem worldwide. While many underlying mechanisms may account for carbapenem resistance in this species, the possession of metallo-β-lactamase (MBL) genes is of particular concern because these enzymes are able to hydrolyze all β-lactam antimicrobials with the exception of aztreonam. In addition, these genes may be mobilized and transferred between different species of bacteria. We conducted a study in 2008 to investigate if there were any changes in the epidemiology of *P. aeruginosa* isolates containing MBL genes in our hospital compared to results from an earlier survey carried out in 2000 (3).

Of 2,552 nonduplicate *P. aeruginosa* organisms isolated in 2008, 123 isolates were imipenem resistant. Of these, 11 were positive for MBL production by imipenem-EDTA disk diffusion (5). Nine of these yielded a product by multiplex PCR for MBL genes (2). The individual MBL genes were then amplified and sequenced. The clonal relationship between isolates with MBL genes was determined by pulsed-field gel electrophoresis (PFGE) of chromosomal DNA restricted with SpeI (3). The PFGE band patterns were analyzed with Bionumerics (Applied Maths NV, Sint-Martens-Latem, Belgium), and all strains with more than 85% similarity were considered to belong to the same clone. All strains were further subjected to multilocus sequence typing (MLST) (1). Because it is a nucleic acid sequence-based method, MLST is able to characterize bacterial types in an unambiguous fashion and establish evolutionary relationships between strains better than band-based methods like PFGE. Representative MBL-producing *P. aeruginosa* isolates from the 2000 survey were also subjected to PFGE and MLST. MLST profiles were submitted to eBURST V3 (http://eburst.mlst.net/) on 10 March 2010. Isolates sharing six out of seven alleles were assigned to the same BURST group and can be considered to belong to the same clonal complex descended from a common founder genotype. The PFGE, MBL gene sequence, and MLST results are summarized in Fig. 1.

In our previous study, 21 of 2,094 (1.0%) of all nonduplicate *P. aeruginosa* isolates in our hospital had MBL genes (3). With the exception of one isolate with bla_{IMP-7}, all other isolates had bla_{IMP-1} and belonged to one of two PFGE clones. Isolates belonging to clone A had sequences identical to that of the original bla_{IMP-1} first reported in Japan. Four representatives of clone A isolated from our hospital in 2000 had sequence type 964 (ST964) by MLST. Isolates belonging to clone B isolated in 2000 had sequences for variant bla_{IMP-1} (bla_{IMP-1v}) with four silent mutations. Three representatives of this clone from 2000 had ST233 and one had ST742 based on MLST. All four representatives of clone B belong to the same BURST group, which was different from that of clone A.

In contrast, in the 2008 survey, 9 of 2,552 (0.35%) nonduplicate *P. aeruginosa* isolates had MBL genes. Unlike the earlier study, there were no large clonal outbreaks. Two isolates with bla_{IMP-1v} had similar PFGE patterns and belonged to the same BURST group as representative isolates from clone B in 2000.

Two isolates from 2008 with bla_{IMP-7} had similar PFGE patterns and shared the same BURST group. The rest of the isolates from 2008 had distinct PFGE patterns.

There was a greater diversity of MBL genes compared to the 2000 survey results. In particular, this is the first time that bla_{VIM-2} and bla_{VIM-6} have been found in *P. aeruginosa* in Singapore. bla_{IMP-26} is a novel MBL gene that differs from bla_{IMP-4} at position 145 (G-to-T change). The translated amino acid sequence differs from IMP-4 at residue 49 (phenylalanine

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▽ Published ahead of print on 12 May 2010.

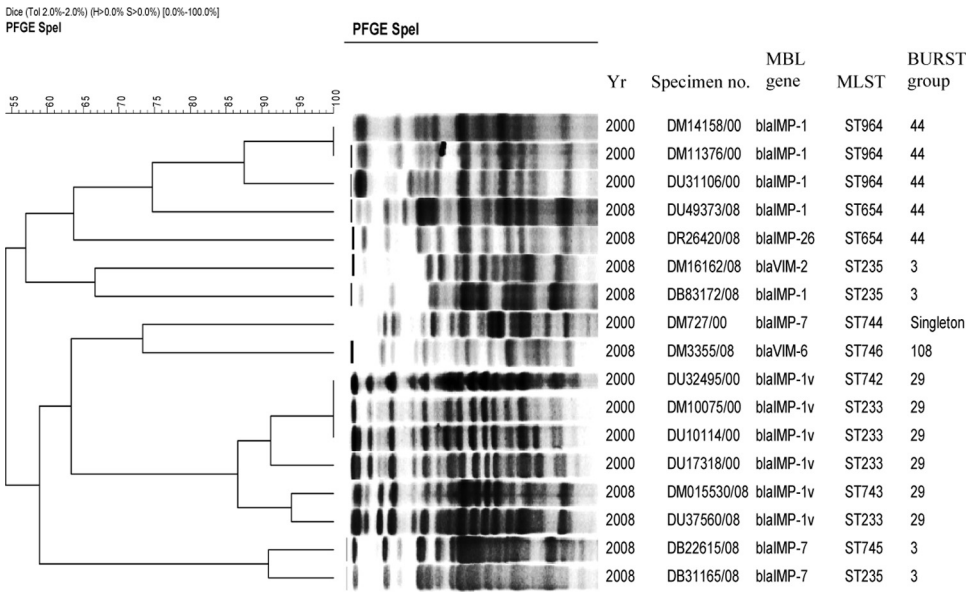

FIG. 1. Dendrogram of PFGE patterns of *P. aeruginosa* isolates with metallo-β-lactamase genes, showing the year of isolation, MLST sequence type, and BURST group.

for valine). This sequence has been previously deposited in the GenBank database as IMP-4 from an *Acinetobacter calcoaceticus* isolate from Malaysia (accession number ABC24668.1).

Three of the isolates in this study (separately containing $bla_{\text{VIM-2}}$, $bla_{\text{IMP-1}}$, and $bla_{\text{IMP-7}}$) belonged to ST235. This sequence type has been described in a VIM-producing *P. aeruginosa* isolate in Belgrade and is the founder of an international clonal complex of isolates bearing MBL genes found in several countries in Europe (6). Recently, an increasing prevalence of IMP-1-producing *P. aeruginosa* has been found in Hiroshima, Japan. This was due entirely to the clonal expansion of only two lineages, ST235 (BURST group 3) and ST357 (BURST group 108) (4). This is similar to the situation that existed in Singapore in 2000, where only two lineages (BURST groups 29 and 44) accounted for the majority of MBL-producing *P. aeruginosa* (3).

It is noteworthy that the original fear that a clone of MBL-producing *P. aeruginosa* would become established in Singapore has not been realized. The BURST group 29 and 44 lineages from 2000 were represented by only one to two isolates in 2008. The two *P. aeruginosa* isolates with $bla_{\text{IMP-7}}$ in 2008 are unrelated to the solitary isolate with $bla_{\text{IMP-7}}$ from 2000. It has been suggested that *P. aeruginosa* displays an epidemic population structure, with a limited number of clones emerging from a large number of unrelated genotypes (7). Although we did not correlate our study with hospital infection control measures, the Japanese data and our own seem to suggest that controlling the prevalence of MBL-producing *P. aeruginosa* may be achieved by preventing the transmission of specific epidemic clones.

While it is reassuring to note that the prevalence of MBL producers in carbapenem-resistant *P. aeruginosa* has not increased, the increased diversity of MBL genes represents a new cause for concern. We were unable to characterize the gene responsible for the MBL phenotype in two isolates in this study, and these may represent novel resistance determinants. Although clones of MBL-producing *P. aeruginosa* have not become established, it seems likely, given the variation of MBL genes and MLST types in this study, that MBL-producing *P. aeruginosa* continues to be introduced to our hospital from diverse sources.

Nucleotide sequence accession number. The sequence for $bla_{\text{IMP-26}}$ was submitted to GenBank under the accession number GU045307.

We acknowledge Ong Lan Huay for technical assistance.

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