Regional dissemination of Acinetobacter species harbouring metallo-β-lactamase genes in Japan

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Abstract

Metallo-β-lactamase (MBL) producers have been reported among the various Acinetobacter species worldwide. In this study, the epidemiology and molecular characteristics of carbapenemase-encoding genes and mobile elements were studied to analyse the regional dissemination of MBL genes in Acinetobacter species. From January 2001 to December 2006, 48 Acinetobacter isolates harbouring MBL genes identified from five hospitals in Kyoto and Shiga Prefecture, Japan were collected and analysed. The partial rpoB gene or the 16S-23S ribosomal RNA intergenic spacer region was sequenced to obtain a species-level identification. Molecular typing using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) was performed. Twenty-five Acinetobacter pittii isolates were divided into eight PFGE types and five sequence types (STs) using MLST. Nine Acinetobacter bereziniae isolates belonged to five PFGE types. Five Acinetobacter nosocomialis isolates were divided into two PFGE types and two STs. Three unclassified Acinetobacter species isolates were divided into two PFGE types. Eighteen of the 25 A. pittii isolates belonged to ST119 and were identified from four hospitals. The blaIMP-19 gene was detected in 41 of 48 isolates, including all of the A. pittii ST119 isolates. The blaIMP-1 and blaIMP-11 genes were detected in four and three isolates, respectively. The MBL genes were all embedded within a class 1 integron as a gene cassette array: blaIMP-19-aac(6')-31-blaOXA-21-aadA1, catB8-like/oacA4-blaIMP-1 and blaIMP-11. This study is the first report demonstrating the regional dissemination of MBL-producing Acinetobacter species. A. pittii ST119 harbouring blaIMP-19 was widely spread throughout the Kyoto-Shiga region.

Keywords: Carbapenemase, class 1 integron, gene cassettes, regional epidemiology, species-level identification

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Introduction

Over the past two decades, the number of carbapenem-resistant Acinetobacter species isolates has dramatically increased, and these organisms have been disseminated worldwide. Metallo-β-lactamases (MBLs) and carbapenem-hydrolysing class D β-lactamases (CHDLs) are common contributors to carbapenem resistance [1]. MBL genes are typically embedded in integrons that accumulate antimicrobial resistance gene cassettes that are associated with the intra- and interspecies transfer of these resistance genes. Insertion sequence (IS) elements, another type of mobile genetic element, may provide promoters that induce overexpression and play a role in the acquisition of CHDL genes [1].

Acinetobacter baumannii strains, especially those belonging to worldwide lineages, such as European clone I, II and III, have been reported to show carbapenem non-susceptibility, which is primarily due to CHDLs [2,3]. IMP-, VIM- and NDM-type MBL genes have been detected among A. baumannii [4]. In addition, MBL genes have also been detected among various Acinetobacter species other than A. baumannii, such as: blaIMP-1, -4, and -8 and blaVIM-2 among Acinetobacter pittii (formerly Acinetobacter genomic species 3); blaIMP-1, and -4 and blaVIM-11 among Acinetobacter nosocomialis (formerly Acinetobacter genomic species 13TU); blaIMP-1, blaVIM-2 and blaIMP-1 among Acinetobacter bereziniae (formerly Acinetobacter genomic species 10); blaIMP-4 among Acinetobacter genomic species 16; blaIMP-1 among Acinetobacter radioresistens; and blaVIM-11 among Acinetobacter junii [5–9].
In a previous study, we identified the dissemination of bla\textsubscript{Imp,19}, which is located in a class I integron in various Acinetobacter species, including A. baumannii, A. pittii, Acinetobacter johnsonii and A. junii [10]. However, this study was only performed at a single institute, and the regional dissemination of MBL genes among Acinetobacter species has not been studied extensively. Therefore, the aim of this study was to analyse the regional epidemiology of MBL genes and their genetic environments among Acinetobacter species.

Materials and Methods

Bacterial isolates and MBL screening
From January 2001 to December 2006, a total of 1713 Acinetobacter species clinical isolates were identified from five hospitals, including three teaching university hospitals (A, B and E) and two city hospitals (C and D) in Kyoto and Shiga Prefecture, Japan. Three hospitals (A, B and C) were located in Kyoto, and two hospitals (D and E) were located in Shiga. The isolates were primarily identified as Acinetobacter species using the VITEK2 system (Sysmex-bioMérieux, Marcy l’Etoile, France) or the MicroScan system (Siemens Healthcare Diagnostics, Tokyo, Japan) in each hospital. Eighty-two of 1713 isolates with resistance to carbapenems or ceftazidime were detected using an automated system through phenotypic MBL screening. A phenotypic MBL screening test was performed using three disks (two Kirby-Bauer disks containing 30 μg of ceftazidime and one disk containing 3 mg of sodium mercaptoacetic acid (Eiken Chemical, Tokyo, Japan)), as previously described [11]. A ≥5 mm difference in the inhibition zone between the disks was considered a positive result for the detection of MBL. Fifty-four MBL-positive isolates of Acinetobacter were sent to Kyoto University Hospital for further analysis. Only one MBL-positive isolate per patient was included in this study. PCR screening was performed for the MBL genes (bla\textsubscript{Imp}, bla\textsubscript{Vim} and bla\textsubscript{NDM-1}) [10]. A total of 48 of the 54 Acinetobacter isolates were positive for MBL genes and were included in this study.

Species-level identification
Species identification was performed through sequencing of the partial rpoB gene (zone I between positions 2916 and 3267, 352 bp), as previously described [12,13]. The nucleotide sequence homology was searched using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). For isolates of the non-Acinetobacter calcoaceticus-Acinetobacter baumannii (ACB) complex, the sequences were compared with the reference sequences of Acinetobacter strains using the neighbour-joining method of the CLUSTAL X program [13]. The intergenic spacer region of 16S-23S ribosomal RNA was sequenced to identify the ACB complex [14].

Molecular typing
Molecular typing was performed using pulsed-field gel electrophoresis (PFGE) with the GenePath system (Bio-Rad, Tokyo, Japan) and the restriction enzyme Apal (New England BioLabs, Tokyo, Japan), as previously described [15]. The pattern analysis was conducted using GelCompar II v.4.062 software (Applied Maths, Sint-Martens-Latem, Belgium) with the unweighted-pair group method based on Dice coefficients and a cut-off of 80% similarity. Multilocus sequence typing (MLST) was also performed on the ACB complex using the Pasteur Institute scheme, and the results were analysed according to the Institut Pasteur MLST Database (http://www.pasteur.fr/recherche/genopole/PPB/mlst/Abau-mannii.html) [16].

Antimicrobial susceptibility testing
Antimicrobial susceptibility was determined using the broth microdilution method with commercially manufactured plates (Eiken Chemical) according to the 2012 CLSI guidelines and breakpoint criteria [17,18].

PCR amplification of carbapenemase-encoding genes, integrase genes and ISs
PCR screening was performed for the carbapenemase-encoding genes and mobile genetic elements, including the CHDL genes (bla\textsubscript{OXA-23-like}, bla\textsubscript{OXA-24-like}, bla\textsubscript{OXA-51-like} and bla\textsubscript{OXA-58-like}), integrase genes (intI1, intI2 and intI3) and IS element genes (IS\textsubscript{Ab1}, IS\textsubscript{Ab2} and IS\textsubscript{Ab3}), in addition to MBL genes, as previously described [10].

Analysis of a class I integron carrying MBL genes and the genetic environment of CHDL genes
For the detection of class I integron gene cassettes, PCR amplification (integron PCR) was performed with primers directed toward the 5’-conserved segments (CSs) and 3’-CS using PrimeSTAR\textsuperscript{™} HS DNA polymerase (TaKaRa Bio, Otsu, Japan) (Fig. 1). The PCR procedures, cloning and sequence analysis of class I integron cassettes have been previously described [10]. For analysis of class I integrons carrying bla\textsubscript{Imp,19}, cloning and sequencing were conducted for five clinical isolates and other strains harbouring bla\textsubscript{Imp,19} using PCR mapping with the newly designed primer sets (Fig. 1) [19]. Cloning and sequencing were also performed to identify the genetic arrays of class I integrons carrying bla\textsubscript{Imp,1} and bla\textsubscript{Imp,11}. To determine the genetic structures of the regions that flank the CHDL genes, a PCR analysis was performed using a series of previously described primers.
has been previously reported for the identification of bacter species using the partial isolates were identified from four hospitals (A, B, D and E), formerly (formerly

guillouiae identified as A. nosocomialis
guillouiae region. Twenty-five isolates were identified as that harboured the MBL gene were identified in the Kyoto-Shi-

For this study, 48 clinical isolates of the Bacterial isolates and species identification

Results and Discussion

Nucleotide sequence accession numbers

The nucleotide sequences of the integron gene cassettes that are newly reported in this paper have been submitted to the DNA Data Bank of Japan (DDBJ) and have been assigned the accession numbers AB713931, AB713932, AB713933, AB713952, AB713953 and AB713954.

Results and Discussion

Bacterial isolates and species identification

For this study, 48 clinical isolates of the Acinetobacter species that harboured the MBL gene were identified in the Kyoto-Shiga region. Twenty-five isolates were identified as A. pittii, nine isolates were identified as A. bereziniae, five isolates were identified as A. nosocomialis, and three isolates were unclassified Acinetobacter species. Single isolates of A. baumannii, Acinetobacter guillouiae (formerly Acinetobacter genomic species 11), Acinetobacter genomic species 15TU, Acinetobacter genomic species 16, A. junii and Acinetobacter ursingii were also identified. Fig. 2 illustrates the phylogenetic relationships between non-ACB complex isolates and Acinetobacter reference strains. A total of three Acinetobacter species isolates exhibited c. 96% identity to the rpoB gene in Acinetobacter gyllenbergi and belonged to the same species with high bootstrap values in the phylogenetic tree. However, these three isolates had a low bootstrap value (49.2%) for the corresponding branch of A. gyllenbergi. Therefore, we defined these isolates as ‘unclassified’ Acinetobacter species. The presence of unclassified species has been previously reported for the identification of Acinetobacter species using the partial rpoB gene sequence [13]. A. pittii isolates were identified from four hospitals (A, B, D and E), A. bereziniae isolates were identified from three hospitals (A, B and C), A. nosocomialis isolates were identified from three hospitals (A, B and D) and unclassified Acinetobacter species were identified from two hospitals (A and B) (Table 1). The origins of A. pittii, A. bereziniae, A. nosocomialis and unclassified Acinetobacter species isolates are shown in Fig. 3. The origins of other isolates were obtained as follows: one A. baumannii (strain A12) was recovered from a nasopharyngeal swab; one A. guillouiae isolate (A13) was recovered from bile; single isolates of A. genomic species 16 (A5) and A. genomic species 15TU (A9) were recovered from ascites; a single A. junii isolate (E7) was recovered from sputum; and a single A. ursingii isolate (A7) was recovered from blood.

The genus Acinetobacter currently comprises 27 validly named species and several other genomic species with provisional designations [21–23]. Using PCR-based species-level identification [12,14], we identified Acinetobacter species harbouring MBL genes that had not previously been reported (e.g. A. guillouiae, genomic species 15TU and A. ursingii). These identification and molecular typing methods are necessary for developing a detailed epidemiology of MBL genes among non-A. baumannii Acinetobacter species.

Molecular typing

Fig. 3 shows the results of molecular typing using PFGE and MLST. The 25 A. pittii isolates were divided into eight PFGE types and five sequence types (STs) using MLST (ST63, ST64, ST93, ST119 and ST121) (Fig. 3a). ST119 was identified in closely related A. pittii isolates from four hospitals (A, B, D and E) (Table 1). The A. pittii non-ST119 isolates were identified from two hospitals (A and B), but only one clonal PFGE type was identified in the ST63 isolates. The other isolates were sporadically identified (Fig. 3a). The nine A. bereziniae isolates were primarily identified from two hospitals (A and B) and could be divided into five PFGE types; the PFGE
FIG. 2. Neighbour-joining tree based on the partial rpoB gene (zone 1) sequence from isolates containing the non-ACB complex and reference strains of the *Acinetobacter* species. The cluster analysis was conducted using the CLUSTAL X program. The GenBank accession numbers are shown in parentheses. The numbers at the branch points indicate the bootstrap values (% based on 1000 resamplings). Bar, 1% sequence divergence.
Institute scheme. Thus, we conducted MLST using the Pasteur number of sequence types submitted (http://pubmlst.org/
might be more applicable than the Bartual scheme for the
A. baumannii
comparison with
A. pittii
epidemiologies of
A. pittii
isolate belonging to ST120 was not related
to European clones I, II or III.
The molecular epidemiologies of the Acinetobacter isolates
harbouring MBL genes are lacking. Although the molecular
epidemiologies of A. pittii are currently insufficient for com-
parison with A. baumannii, the Pasteur Institute MLST scheme
might be more applicable than the Bartual scheme for the
number of sequence types submitted (http://pubmlst.org/
abaumannii). Thus, we conducted MLST using the Pasteur
Institute scheme.

Antimicrobial susceptibility
The antimicrobial susceptibility profiles are shown in Table 2.
None of the isolates were susceptible to imipenem. How-
ever, three isolates of A. pittii ST119 and one isolate of
A. baumannii were susceptible to meropenem. Most of the
isolates were susceptible to minocycline, colistin and ampicil-
lin-sulbactam. However, ampicillin-sulbactam and colistin
exhibited lower susceptibility rates against A. bereziniae,
A. nosocomialis and unclassified Acinetobacter species than
against other Acinetobacter isolates. In particular, all three of
the unclassified Acinetobacter species isolates were resistant
to colistin. The aminoglycosides had lower susceptibility
rates against A. pittii and A. nosocomialis species
than against other Acinetobacter isolates.

Carbapenemase-encoding genes and the genetic environ-
ment
All of the isolates harboured the class 1 integrase gene (intI1).
Table 2 outlines the distribution of MBL genes among the
Acinetobacter species. The blaIMP-19 gene was predominantly
detected in Acinetobacter isolates, including 23 isolates of
A. pittii (all 18 isolates of ST119) and all isolates of A. bereziniae,
A. nosocomialis, A. baumannii, A. guillouiae, genomic species 16
and A. junii. The blaIMP-1 gene was detected in four isolates
(A. pittii, unclassified Acinetobacter species, genomic species
15TU and A. ursingii) and blaIMP-11 was detected in three
isolates (A. pittii and two unclassified Acinetobacter species
isolates).

CHDL genes primarily contribute to carbapenem non-sus-
ceptibility among Acinetobacter species [2,24]. Even in Japan,
a recent study showed that 94.5% of the carbapenem-resistant
Acinetobacter isolates harboured CHDL genes, and only one
A. pittii isolate harboured an IMP-type MBL gene [25]. How-
ever, in another study conducted in Asia, A. pittii was reported
to harbour MBL genes, including blaIMP-1, -4, and -8 and blaVIM-2
[6,7,9].

All of the MBL genes detected in this study were embed-
ded in a class 1 integron. The blaIMP-19 gene was identified in
a gene cassette array of blaIMP-19-aac(6')-31-blaOXA-21-aadA1,
the blaIMP-1 gene was in an array of catB8-like/aacA4-blaIMP-1,
and blaIMP-11 was the only component of another gene casset-
type array (Fig. 1). The same class 1 integron carrying blaIMP-
19 was detected in various Acinetobacter species. While this
finding was reported in our previous study, the identification
of this integron in A. nosocomialis, A. bereziniae and A. guillo-
uae was a recent discovery. These findings provide strong
evidence for the inter- and intraspecies dissemination of this
class 1 integron among Acinetobacter species. In contrast,
no evidence of the intraspecies dissemination of a class 1 integ-
tron carrying blaIMP-1 and blaIMP-11 was demonstrated,
although the regional spread and interspecies dissemination
of these two MBL genes was detected.

One isolate of A. baumannii ST120 harboured blaOXA-51-like
and ISAba2 in addition to blaIMP-19, but ISAba2 was not identi-
fied up- or downstream of blaOXA-51-like. ISAba3 flanked both
sides of the blaOXA-58 gene, which was detected in the single
isolate of A. pittii ST93 harbouring blaIMP-1 and three isolates
(one isolate of A. pittii ST63 and two isolates of unclassified
Acinetobacter species) harbouring blaIMP-11 (Fig. 3). Other
CHDL genes were not detected in the isolates included in

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**TABLE 1. Numbers of isolated Acinetobacter strains**

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>Total</th>
<th>Hospitals</th>
</tr>
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<tbody>
<tr>
<td>A. pittii (ST119)</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td>15</td>
<td>A, B, D, E</td>
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<tr>
<td>A. pittii (non-ST119)</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td>11</td>
<td>A, B, C</td>
</tr>
<tr>
<td>A. bereziniae</td>
<td>3</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td>9</td>
<td>A, B, D</td>
</tr>
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<td>A. nosocomialis</td>
<td>7</td>
<td></td>
<td>4</td>
<td>1</td>
<td>4</td>
<td></td>
<td>16</td>
<td>A, B, E</td>
</tr>
<tr>
<td>Unclassified species</td>
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<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>4</td>
<td>A, E</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>8</td>
<td>11</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

*The non-ST119 isolates include ST63, ST64, ST93 and ST121.
*Other strains include A. baumannii, A. guillouiae, genomic species 1STU, genomic species 16, A. junii and A. ursingii.
*Hospitals where each species was identified.
This study. This finding is consistent with previous reports [7,26]. Among the non-
A. baumannii Acinetobacter species, two ISAb3 genes flanked the blaOXA-58-like gene, which is
widely disseminated in Eastern Asian countries.

Notably, there was one major limitation of this study. We screened the Acinetobacter isolates harbouring MBL genes collected between 2001 and 2006 and could not directly
determine whether the MBL genes described in this study

### Table 1. IS and MBL gene co-occurrence among Acinetobacter species isolates.

<table>
<thead>
<tr>
<th>ID</th>
<th>Year</th>
<th>Hospital</th>
<th>Specimen</th>
<th>MBL genes</th>
<th>CHDL genes</th>
</tr>
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<tbody>
<tr>
<td>121</td>
<td>A16</td>
<td>2004</td>
<td>Swab*</td>
<td>IMP-19</td>
<td>-</td>
</tr>
<tr>
<td>121</td>
<td>A2</td>
<td>2001</td>
<td>Urine</td>
<td>IMP-19</td>
<td>-</td>
</tr>
<tr>
<td>91</td>
<td>B15</td>
<td>2004</td>
<td>Unknown</td>
<td>IMP-1</td>
<td>OXA-58</td>
</tr>
<tr>
<td>64</td>
<td>B2</td>
<td>2001</td>
<td>Blood</td>
<td>IMP-19</td>
<td>-</td>
</tr>
<tr>
<td>63</td>
<td>A3</td>
<td>2001</td>
<td>Urine</td>
<td>IMP-19</td>
<td>-</td>
</tr>
<tr>
<td>63</td>
<td>A4</td>
<td>2001</td>
<td>Urine</td>
<td>IMP-19</td>
<td>-</td>
</tr>
<tr>
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<td>A17</td>
<td>2005</td>
<td>Swab*</td>
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<td>OXA-58</td>
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<tr>
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<td>E4</td>
<td>2005</td>
<td>E</td>
<td>Urine</td>
<td>IMP-19</td>
</tr>
<tr>
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<td>2005</td>
<td>E</td>
<td>Urine</td>
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</tr>
<tr>
<td>119</td>
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<td>2005</td>
<td>E</td>
<td>Bile</td>
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<tr>
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<td>E</td>
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<td>B7</td>
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</tr>
<tr>
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<td>B9</td>
<td>2003</td>
<td>B</td>
<td>Blood</td>
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<tr>
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<td>B16</td>
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<td>Unknown</td>
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<tr>
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<td>Urine</td>
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</tr>
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<td>B</td>
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</tr>
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<td>A18</td>
<td>2006</td>
<td>A</td>
<td>Sputum</td>
<td>IMP-19</td>
</tr>
<tr>
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<td>E5</td>
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<td>E</td>
<td>Urine</td>
<td>IMP-19</td>
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<tr>
<td>119</td>
<td>A11</td>
<td>2004</td>
<td>A</td>
<td>Wound</td>
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<td>119</td>
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<td>Urine</td>
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<td>119</td>
<td>B9</td>
<td>2003</td>
<td>B</td>
<td>Blood</td>
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<td>2004</td>
<td>E</td>
<td>Sputum</td>
<td>IMP-19</td>
</tr>
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</table>

### Figure 3. The results of the PFGE analysis with Apal, MLST, and the profiles of carbapenemase-encoding genes. The results shown in panel (a) were obtained from 25 A. pittii isolates, the results in panel (b) were obtained from nine A. bereziniae isolates, the results in panel (c) were obtained from five A. nosocomialis isolates, and the results shown in panel (d) were obtained from three unclassified Acinetobacter species isolates. The STs were based on a Pasteur Institute scheme. The MLST was performed using only the A. calcoaceticus-A. baumannii complex. The MBL genes and CHDL gene columns indicate the types of carbapenemase-encoding genes harboured. *Swab: nasopharyngeal swab. ST, sequence type; MBL, metallo-β-lactamase; CHDL, carbapenem-hydrolysing class D β-lactamase.

This finding is consistent with previous reports [7,26]. Among the non-
A. baumannii Acinetobacter species, two ISAb3 genes flanked the blaOXA-58-like gene, which is
widely disseminated in Eastern Asian countries.
TABLE 2. Susceptibilities of Acinetobacter species harbouring MBL genes and the distribution of MBL genes among these species

<table>
<thead>
<tr>
<th></th>
<th>No. (%) of isolates:</th>
<th></th>
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<td></td>
<td>A. pittii (ST119)</td>
<td>A. pittii (non-ST119)</td>
</tr>
<tr>
<td>Antimicrobial susceptibility</td>
<td>n = 18</td>
<td>n = 7</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>17 (94.4)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>9 (50.0)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>3 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>14 (77.8)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>15 (83.3)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>17 (94.4)</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6 (33.3)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>16 (88.9)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Minocycline</td>
<td>17 (94.4)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>0 (0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Colistin</td>
<td>17 (94.4)</td>
<td>7 (100)</td>
</tr>
</tbody>
</table>

*The non-ST119 isolates include ST63, ST64, ST93 and ST121.

*Other strains include A. baumannii, A. guillouiae, genomic species 15TU, genomic species 16, A. junii and A. ursingii.

were still disseminated among Acinetobacter species. From 2007 to 2010, 10 Acinetobacter isolates harboured exactly the same class 1 integron containing \( \text{bla} \) IMP-19 as identified in our institute [10]. These data suggest the possibility of continued integron-borne MBL gene dissemination among the species in this region.

In conclusion, this is the first report that demonstrates the regional and molecular epidemiology of Acinetobacter species harbouring integron-borne MBL genes and provides details of their species-level identification. The IMP-type MBL genes were detected in various Acinetobacter species. In particular, the \( \text{bla}_{\text{IMP-19}} \) gene was predominately identified among many Acinetobacter species, particularly A. pittii ST119. The CHDL genes were indeed major contributors to carbapenem-non-susceptibility worldwide; however, the continued investigation of MBL genes in Acinetobacter species is required.

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Authors’ Contributions

M. Yamamoto conceived, designed and conducted the genetic studies and drafted the manuscript. S. Ichiyama participated in manuscript preparation. M. Nagao, Y. Matsumura, G. Hotta, A. Matsushima, Y. Ito and S. Takakura participated in the genetic studies.

Transparency Declarations

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primers used for the PCR analysis of integrons and ISs in this study.

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References


