Molecular epidemiology of carbapenem-non-susceptible Acinetobacter baumannii in Japan

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Objectives: Acinetobacter baumannii presents a clinical challenge when it is non-susceptible to carbapenems. The prevalence of carbapenem-non-susceptible A. baumannii in Japan is unclear, as previous studies have been limited in scope. We investigated the spread of carbapenem-non-susceptible A. baumannii in Japan and performed a comparison with findings from overseas.

Methods: A total of 305 non-duplicate clinical isolates of Acinetobacter spp. from 176 medical facilities in all geographical regions of Japan were tested for susceptibility to antimicrobial agents by the agar dilution method. Isolates with MICs of imipenem $\geq$ 4 mg/L underwent PCR analysis of OXA-type $\beta$-lactamase gene clusters and metallo-$\beta$-lactamase genes. These isolates were further analysed by sequencing of OXA-type $\beta$-lactamases and by multilocus sequence typing (MLST).

Results: Fifty-five of the 305 clinical isolates had MICs of imipenem $\geq$ 4 mg/L. The OXA-51-like carbapenemase gene was detected in 52 of these 55 isolates. Within the OXA-51-like gene cluster, OXA-66 was found in 43 (82.7%) of the 52 isolates. MLST identified the following sequence types (STs): ST74, ST76, ST92, ST106, ST188 and ST195 in 2 (3.8%), 2 (3.8%), 40 (76.9%), 5 (9.6%), 2 (3.8%) and 1 (1.9%) of the isolates, respectively. In particular, ST92 was found in 31 (91.2%) of the 34 A. baumannii isolates with MICs of imipenem $\geq$ 16 mg/L.

Conclusions: This is the first report on the molecular epidemiology of A. baumannii with MICs of imipenem $\geq$ 4 mg/L in Japan. OXA-66 and ST92 were dominant among these isolates.

Keywords: OXA-66, MLST, ST92, CC92

Introduction

Acinetobacter baumannii represents the most clinically important and frequently detected member of the Acinetobacter species, with the respiratory tract being the source for approximately half of all isolates. Although there are a few reports that the OXA-51-like gene was detected in non-baumannii Acinetobacter species recently in Taiwan, the Amber class D OXA-51-like gene is intrinsic to A. baumannii and has been found to be a method of its identification.

A. baumannii infections usually occur in the most vulnerable hospitalized patients, and commonly include bloodstream infections, ventilator-associated pneumonia and urinary tract infections. On a global basis, A. baumannii has emerged as a major healthcare facility-associated pathogen in recent years, with outbreaks being reported among vulnerable patients such as those in intensive care units. Outside Japan, carbapenem-non-susceptible A. baumannii has become one of the most troublesome pathogens in medical institutions.

Of particular concern has been the emergence of carbapenem-non-susceptible A. baumannii by acquisition of carbapenem-hydrolysing OXA-type enzymes, especially when isolates are multidrug resistant, making antimicrobial choices quite limited.

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While there are country-specific variations of prevalence rates, a global multicentre surveillance study, the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Europe 2007 showed that the susceptibility of imipenem for Acinetobacter spp. was 83.3% in 2004 and 78.9% in 2007, while the susceptibility rate was reported to be only 60.2% in the USA (2004–05). \(^3\)

In addition, widespread dissemination of carbapenemases among Acinetobacter spp. in Asia-Pacific nations has been demonstrated by the SENTRY Surveillance Program, with imipenem susceptibility being found in 73.7% of isolates during the period from 2001 to 2004; this rate fell to 52% in 2006–07. \(^4\)

While it is clear that global spread of carbapenem-non-susceptible A. baumannii has occurred, the epidemiology in Japan remains unclear because this country does not participate in SENTRY and a nationwide survey has not been performed so far.

In the present study we investigated 305 Acinetobacter spp. isolates from patients in 176 medical institutions throughout the different geographical regions of Japan in order to better understand the spread of carbapenem-non-susceptible A. baumannii.

Materials and methods

**Bacterial strains**

A total of 305 Acinetobacter spp. isolates were obtained from clinical specimens between November 2009 and March 2010 at 176 medical institutions (116 hospitals and 60 clinics) in different geographical regions of Japan. These 305 non-duplicate Acinetobacter spp. isolates were identified at the genus level by BML Biomedical Laboratories R&D Center (Kawagoe, Saitama, Japan) using the Microscan Walkaway System (Siemens Healthcare Diagnostics Japan, Tokyo, Japan) and the Vitek 2 System (SystemsbioMérieux Japan, Kobe, Japan). Identification of A. baumannii was confirmed by PCR amplification of the OXA-51-like gene. In addition, non-A. baumannii was identified at the species level by partial RNA polymerase β-subunit (rpoB) gene sequencing. \(^5\)

**Antimicrobial susceptibility testing**

MICs were determined and interpreted according to the methods and breakpoints defined by the CLSI. \(^6\) The agar dilution method was used for the following agents: ampicillin (Sigma-Aldrich, Osaka, Japan), ampicillin/sulbactam (Pfizer, New York City, New York, USA), cefotaxin (Sigma-Aldrich), cefpodoxime (Daiichi Sankyo, Tokyo, Japan), cefoxitin (Sigma-Aldrich), ceftazidime (Sigma-Aldrich), cefepime (Bristol-Myers Squibb, Tokyo, Japan), imipenem (Banyu Pharmaceutical, Tokyo, Japan), meropenem (Dainippon Sumitomo Pharma, Osaka, Japan), gentamicin (Sigma-Aldrich), amikacin (Sigma-Aldrich), ciprofloxacin (Bayer Healthcare, Osaka, Japan) and levofloxacin (Daichi Sankyo). The range of concentrations tested was 0.125–128 mg/L. Quality control strains were Escherichia coli ATCC 25922 and E. coli ATCC 35218.

**Carbapenemase analysis by PCR**

Isolates with MICs of imipenem ≥ 4 mg/L were further characterized by PCR using the GeneAmp PCR System 9600R (Roche Molecular System, Pleasanton, CA, USA). PCR amplification was done for detection of OXA-51-like, OXA-23-like, OXA-24-like, ISAbA1/blAOXA-51-like complex, ISAbA1/blAOXA-23-like complex and metallo-β-lactamase (MBL) genes (including IMP-1, IMP-2, VIM-1, VIM-2 and SIM) as previously reported. \(^7\)

**Sequencing of OXA-type β-lactamase and MBL genes**

Sequencing of OXA-type β-lactamase and MBL genes was performed according to the method of Pournaras et al. \(^7\) PCR amplicons were purified with a QIA quick PCR Purification Kit (Qiagen K. K., Hilden, Germany), followed by DNA sequencing using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the ABI3730xl Analyzer (Applied Biosystems). BLAST version 2.2.24 was used for sequence analysis (http://blast.ddbj.nig.ac.jp/top-j.html).

**Multilocus sequence typing (MLST) analysis**

Using seven housekeeping genes (gltA, gyrB, gdhB, recA, cpn60, gpi and rpoD), MLST was performed according to the method of Burtual et al. \(^8\) PCR products were purified with a QIA quick PCR Purification Kit, followed by sequencing using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI3730xl Analyzer. DNA sequence variations were analysed using an MLST database for A. baumannii (http://pubmlst.org/abaumannii).

**Results and discussion**

Of the 305 Acinetobacter spp. isolates analysed, 136 (44.6%) were recovered from sputum specimens, 55 (18%) were from urine and 51 (16.7%) were from throat swabs. None was from blood cultures. Agar dilution tests using CLSI breakpoints \(^9\) showed an imipenem-non-susceptible rate of 13.4% (Table 1). This overall imipenem-non-susceptible rate for Acinetobacter spp. was lower than previously reported outside Japan. \(^1\) With regard to multidrug resistance of Acinetobacter spp., a report from the USA indicated the rapid emergence of such resistance, with 60% of isolates being resistant to three or more antimicrobial agents and 30% showing resistance to four or more agents. \(^9\) In contrast, only 8.9% of the isolates displayed resistance to three or more antimicrobial agents in the present study (data not shown). Despite the heavy use of carbapenems in Japan, our findings indicate that carbapenem-non-susceptible isolates remain relatively uncommon compared with their global prevalence.

Of the 305 isolates of Acinetobacter spp., 251 (82.3%) possessed the OXA-51-like gene. In particular, the OXA-51-like carbapenemase gene was detected in 52 of the 55 isolates with MICs of imipenem ≥ 4 mg/L, while only 1 isolate had both OXA-51-like and OXA-23-like genes. All three isolates not possessing OXA-51-like carbapenemase were identified as Acinetobacter pittii by rpoB gene sequencing. Thus, the presence of the ISAbA1/blAOXA-51-like complex was confirmed in almost all imipenem-non-susceptible (MICs of imipenem ≥ 8 mg/L) A. baumannii (35/38 isolates), while absence of the ISAbA1/blAOXA-51-like complex was confirmed in almost all A. baumannii with MICs of imipenem of 4 mg/L (13/14 isolates). None of the A. baumannii isolates possessed IMP, VIM or SIM, while only one A. pittii isolate had the blAOXPIMP gene. Sequencing of the blAOXA-51-like gene cluster showed that 43 (82.7%) of 52 isolates had OXA-66, 5 (9.6%) had OXA-80, 2 (3.8%) had OXA-83, 1 (1.9%) had OXA-51 and 1 (1.9%) had OXA-70. Thus, we found that OXA-66 is dominant in Japan. Sequencing of the IMP gene revealed only IMP-19. Only one isolate (TU1412) had both OXA-66 and OXA-23. This isolate did not possess the ISAbA1/blAOXA-51-like complex, but had the ISAbA1/blAOXA-23-like complex. Unlike the chromosomal blAOXA-51-like genes, mobilization of the OXA-23-like...
Carbapenemase gene via plasmids has resulted in worldwide dissemination of \( \textit{bla} \) \textit{OXA-23-like} in \textit{A. baumannii}. For example, the first South Korean case of \textit{A. baumannii} carrying the \( \textit{bla} \) \textit{OXA-23-like} gene was reported in 2005, but such isolates were detected throughout the country by 2006.\(^{10}\) The SENTRY Surveillance Program report covering the period from 2006 to 2007 in the Asia-Pacific region (excluding Japan) also indicated a high prevalence of class D carbapenemase-encoding genes, mainly \( \textit{bla} \) \textit{OXA-23}, in \textit{A. baumannii}.\(^{4}\) Although our study suggested that the \textit{OXA-23} carbapenemase gene is not yet widespread among \textit{A. baumannii} in Japan, there is the potential for antibiotic selection pressure and plasmid-mediated dissemination of \textit{OXA-23} to mirror the South Korean situation in the near future. Therefore, we plan to continue molecular epidemiological surveillance of \textit{A. baumannii} in Japan.

In this study we did not investigate other carbapenem-resistant mechanisms, such as porins and efflux pumps. Therefore, the mechanism of reducing carbapenem susceptibility in these isolates may involve a synergistic interaction with carbapenemase, efflux overexpression and reduced expression of an outer membrane protein.

When 52 \textit{A. baumannii} strains with MICs of imipenem \( \geq 4 \) mg/L were typed by MLST according to the method of Bartual et al.,\(^{8}\) 40 isolates (76.9\%) were sequence type (ST) 92, 5 isolates (9.6\%) were ST106, 2 isolates (3.8\%) were ST74, 2 isolates (3.8\%) were ST76, 1 isolate (1.9\%) was ST188 and 1 isolate (1.9\%) was ST195 (TU1412; \textit{OXA-23}-producing isolate). However, 31 (91.2\%) of 34 \textit{A. baumannii} isolates with MICs of imipenem \( \geq 16 \) mg/L were ST92, while the prevalence of ST92 declined to 50\% in \textit{A. baumannii} isolates with MICs of 8 mg/L or MICs of 4 mg/L (Table 2). ST92, ST106, ST74 and ST76 belong to clonal complex 92 (CC92), which is the most widely disseminated complex worldwide. Thus, our findings show that global CC92 is also circulating in Japan and that ST92 is the predominant lineage among imipenem-non-susceptible isolates in this country.

In conclusion, this was the first study to characterize imipenem-non-susceptible \textit{A. baumannii} isolates by MLST in Japan in order to better understand the distribution of lineages within this country as well as the molecular epidemiology of \textit{A. baumannii} in comparison with that for the Asia-Pacific region and globally.

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Transparency declarations
None to declare.

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