Antimicrobial susceptibility and mechanisms of high-level macrolide resistance in clinical isolates of Moraxella nonliquefaciens

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We investigated antimicrobial susceptibility and the molecular mechanism involved in conferring high-level macrolide resistance in 47 clinical isolates of Moraxella nonliquefaciens from Japan. Antimicrobial susceptibility was determined using Etest and agar dilution methods. Thirty-two erythromycin-non-susceptible strains were evaluated for the possibility of clonal spreading, using PFGE. To analyse the mechanism related to macrolide resistance, mutations in the 23S rRNA gene and the ribosomal proteins, and the presence of methylase genes were investigated by PCR and sequencing. The efflux system was examined using appropriate inhibitors. Penicillin, ampicillin, amoxicillin, cefixime, levofloxacin and antimicrobials containing β-lactamase inhibitors showed strong activity against 47 M. nonliquefaciens isolates. Thirty-two (68.1 %) of the 47 isolates showed high-level MICs to macrolides (MIC ≥128 mg L−1) and shared the A2058T mutation in the 23S rRNA gene. The geometric mean MIC to macrolides of A2058T-mutated strains was significantly higher than that of WT strains (P<0.0001). Thirty-two isolates with high-level macrolide MICs clustered into 30 patterns on the basis of the PFGE dendrogram, indicating that the macrolide-resistant strains were not clonal. In contrast, no common mutations of the ribosomal proteins or methylase genes, or overproduction of the efflux system were observed in A2058T-mutated strains. Moreover, of the 47 M. nonliquefaciens strains, 43 (91.5 %) were bro-1 and 4 (8.5 %) were bro-2 positive. Our results suggest that most M. nonliquefaciens clinical isolates show high-level macrolide resistance conferred by the A2058T mutation in the 23S rRNA gene. This study represents the first characterization of M. nonliquefaciens.

INTRODUCTION

Moraxella nonliquefaciens, a Gram-negative coccobacillus, is part of the normal microbiota of the human respiratory tract (Vaneechoutte et al., 2012). Previous reports have suggested that M. nonliquefaciens causes various infectious diseases, such as endocarditis, endophthalmitis and pneumonia (Davis et al., 2004; Laukeland et al., 2002; Rafiq et al., 2011), and it has been reported to show susceptibility to various antimicrobials, including macrolides (Davis et al., 2004; Laukeland et al., 2002; Vaneechoutte et al., 2012).

Macrolides act by binding to the 23S rRNA gene and prevent elongation of the peptide chain (Leclercq & Courvalin, 1998). Currently, clarithromycin and azithromycin are frequently used for the treatment of respiratory tract infections (Grover et al., 2012; Higashi & Fukuhara, 2009; Kawai et al., 2012). Mechanisms of resistance against these antimicrobials, including in Moraxella catarrhalis, which is among the Moraxella species most frequently isolated from clinical samples, are known to involve active efflux of...
antimicrobials and modification of the ribosomal target by enzymes such as rRNA methylase, or mutations in domain V of the 23S rRNA gene (Chironna et al., 2011; Chisholm et al., 2010; Li et al., 2011; Saito et al., 2012; Tait-Kamradt et al., 2000; Versalovic et al., 1997). Another mechanism, involving mutations in the ribosomal proteins L4 and L22 encoded by rplD and rplV, respectively, has been reported to underlie resistance in some clinical isolates (Beresio et al., 2006; Tait-Kamradt et al., 2000).

There is, however, a need for more data from clinical isolates to assess the risk of emergence of antimicrobial-resistant M. nonliquefaciens strains. Therefore, we investigated the antimicrobial susceptibility of 47 clinical isolates of M. nonliquefaciens and characterized the molecular mechanism related to high-level resistance to macrolides in these strains. We demonstrated for the first time that a point mutation in the 23S rRNA gene confers high-level resistance to macrolides in M. nonliquefaciens.

METHODS

Bacterial strains and culture conditions. A total of 47 M. nonliquefaciens clinical isolates collected at four university hospitals in Tokyo, Japan, from October 2008 to July 2009, and a type strain, M. nonliquefaciens ATCC 17953, were examined in this study. The clinical isolates were obtained from nasopharyngeal specimens of patients with respiratory tract infections. They were identified using the VITEK 2 system (SYSMEX; bioMérieux) and 16S rRNA gene sequencing using primers 10F and 800R (Table 1). M. nonliquefaciens nonliquefaciens clinical isolates collected at four university hospitals in Tokyo, Japan, from October 2008 to July 2009, and a type strain, M. nonliquefaciens ATCC 17953, were examined in this study. The clinical isolates were obtained from nasopharyngeal specimens of patients with respiratory tract infections. They were identified using the VITEK 2 system (SYSMEX; bioMérieux) and 16S rRNA gene sequencing using primers 10F and 800R (Table 1). M. nonliquefaciens clinical isolates were obtained from nasopharyngeal specimens of patients with respiratory tract infections. They were identified using the VITEK 2 system (SYSMEX; bioMérieux) and 16S rRNA gene sequencing using primers 10F and 800R (Table 1). M. nonliquefaciens strains were cultured at 37 °C on tryptone soya agar (TSA) plates containing 5 % sheep blood (Nippon Becton Dickinson) and brain heart infusion (BHI) agar plates (Oxoid) in an atmosphere of 95 % air and 5 % CO2. Cultures were maintained in 10 % skimmed milk and stored at −80 °C until required for subsequent experiments.

Antimicrobial susceptibility testing. The antimicrobial susceptibility of M. nonliquefaciens strains was determined using the Etest method (AB BIODISK) on TSA containing 5 % sheep blood, according to the manufacturer’s recommendations. The following antibiotics were tested by Etest: penicillin, ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, ceftezime and levofloxacin. Antimicrobial susceptibility to erythromycin (Wako Pure Chemical), clarithromycin (Taisho Pharmaceutical), azithromycin (Tokyo Chemical Industry) and josamycin (Astellas Pharma) was determined by the agar dilution method, using BHI agar. MICs of antimicrobials were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria for M. catarrhalis (CLSI, 2010). Staphylococcus aureus ATCC 29213 was used for quality control.

PFGE. Genomic DNA of 32 erythromycin-non-susceptible M. nonliquefaciens strains was prepared in agarose plugs treated with proteinase K (Takara Bio). The DNA was digested with 25 U SpeI (New England Biolabs) and electrophoresis was performed using a CHEF DRII system (Bio-Rad). Fingerprinting II software (Bio-Rad) was used to analyse the DNA restriction patterns and determine their similarity, based on calculation of the Dice similarity coefficient and using the unweighted pair group method with arithmetic mean algorithm.

DNA sequencing of macrolide-resistance targets and detection of methylase and β-lactamase genes. Bacterial genomic DNA was extracted by boiling. The 23S rRNA gene, rplD and rplV were amplified by PCR using the relevant primer sets (Table 1), as follows:

Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Target gene/primer</th>
<th>Sequence (5'→3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16S-10F</td>
<td>GTTGTGATCCTGGCTCA</td>
<td>Saito et al. (2012)</td>
</tr>
<tr>
<td>16S-800R</td>
<td>TACCAGGGTATCTAATCC</td>
<td></td>
</tr>
<tr>
<td>23S rRNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23S-1930F</td>
<td>GGTAGCGAAATTCCTTGCG</td>
<td>Saito et al. (2012)</td>
</tr>
<tr>
<td>23S-2488R</td>
<td>CGCCGTCGATATGAACTCTT</td>
<td>This study</td>
</tr>
<tr>
<td>rplD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L4-24F</td>
<td>TGACGCGTTGAACATAG</td>
<td>Saito et al. (2012)</td>
</tr>
<tr>
<td>L4-589R</td>
<td>CCTCAAAATGTTTGCTGCT</td>
<td></td>
</tr>
<tr>
<td>rplV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L22-32F</td>
<td>CCATTTCCGACAGAAAGTA</td>
<td>Saito et al. (2012)</td>
</tr>
<tr>
<td>L22-325R</td>
<td>CCCCTACTTTAACAAGTTGA</td>
<td></td>
</tr>
<tr>
<td>erm(A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ermA-F</td>
<td>AGCGGTAACACCCCTCTGAG</td>
<td>Nakaminami et al. (2008)</td>
</tr>
<tr>
<td>ermA-R</td>
<td>TAGTGACATTGTCATCTCA</td>
<td></td>
</tr>
<tr>
<td>erm(B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ermB-F</td>
<td>AGTAACGGTACATTTAGTTTAC</td>
<td>Sutcliffe et al. (1996)</td>
</tr>
<tr>
<td>ermB-R</td>
<td>GAAAAGGTACTCAACCAATA</td>
<td></td>
</tr>
<tr>
<td>erm(C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ermC-F</td>
<td>GCTTAATTGTGTTAATGTGCAAT</td>
<td>Sutcliffe et al. (1996)</td>
</tr>
<tr>
<td>ermC-R</td>
<td>TCAAAACATAATAGATAAA</td>
<td></td>
</tr>
<tr>
<td>erm(F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ermF-L</td>
<td>CGGGTCAGCATTTACTAT</td>
<td>Chung et al. (1999)</td>
</tr>
<tr>
<td>ermF-R</td>
<td>GACCTACCTCATAAGA</td>
<td></td>
</tr>
</tbody>
</table>
3 min of initial denaturation at 95°C, 30 cycles each consisting of 30 s at 95°C, 30 s at 53°C and 1 min at 72°C and 3 min of final extension at 72°C. PCR amplicons were sequenced on an ABI PRISM 3100 genetic analyser (Applied Biosystems). DNA sequences were compared with the M. nonliquefaciens ATCC 17953 sequence (GenBank accession no. AB745464 for 23S rRNA, AB745465 for rplD and AB745466 for rplV) using BLAST.

The presence of the methylase genes erm(A), erm(B), erm(C) and erm(F) was screened for using the relevant primer sets (Table 1) as described previously (Chung et al., 1999; Nakaminami et al., 2008; Roberts et al., 1999; Saito et al., 2012; Sutcliffe et al., 1996). S. aureus, Streptococcus pneumoniae and Bacteroides vulgatus were used as positive controls for erm(A) and erm(C), erm(B) and erm(F), respectively.

PCR amplification of bro was performed as described previously (Khan et al., 2010).

**Analysis of the efflux system.** To investigate the role of efflux systems in resistance to macrolides, efflux pump activity was assessed by the addition of two different inhibitors, phenylalanine arginine β-naphthylamide (PAβN; Sigma-Aldrich) and carbonyl cyanide m-chlorophenylhydrazone (CCCP; Nacalai Tesque) to the growth medium at the final concentration of 10 and 0.1 mg l⁻¹. The presence of the methylase genes erm(A), erm(B), erm(C) and erm(F) was screened for using the relevant primer sets (Table 1) as described previously (Chung et al., 1999; Nakaminami et al., 2008; Roberts et al., 1999; Saito et al., 2012; Sutcliffe et al., 1996). S. aureus, Streptococcus pneumoniae and Bacteroides vulgatus were used as positive controls for erm(A) and erm(C), erm(B) and erm(F), respectively.

**Statistical analysis.** A Mann–Whitney U test was used to determine statistical differences in MICs. In all analyses, P<0.05 was considered significant.

**RESULTS**

**Antimicrobial susceptibility testing**

The results of antimicrobial susceptibility testing of 47 clinical isolates to 12 antibiotics are shown in Table 2. The MIC of antibiotics for an M. liquefaciens type strain ATCC 17953 were as follows: penicillin, 0.040 mg l⁻¹; ampicillin, ≤0.016 mg l⁻¹; amoxicillin, ≤0.016 mg l⁻¹; ampicillin–sulbactam, ≤0.016 mg l⁻¹; amoxicillin–clavulanic acid, ≤0.016 mg l⁻¹; cefixime, ≤0.016 mg l⁻¹; levofloxacin, 0.064 mg l⁻¹; erythromycin, ≤0.125 mg l⁻¹; clarithromycin, ≤0.125 mg l⁻¹; azithromycin, ≤0.125 mg l⁻¹; josamycin, 1 mg l⁻¹.

The MIC₉₀ values of antibiotics, excluding macrolides, for the isolates in this study were: penicillin, 4 mg l⁻¹; ampicillin, 0.25 mg l⁻¹; amoxicillin, 0.5 mg l⁻¹; cefixime, 0.25 mg l⁻¹; levofloxacin, 0.064 mg l⁻¹. The MIC₉₀ values of ampicillin and amoxicillin containing a β-lactamase-inhibitor were 0.125 and 0.125 mg l⁻¹, respectively. These antibiotics showed strong activity against M. nonliquefaciens.

In contrast, macrolides were less active against M. nonliquefaciens than the other seven tested antibiotics: the MIC₉₀ values of erythromycin, clarithromycin, azithromycin and josamycin were >512, 512, 512 and 256 mg l⁻¹, respectively. Based on the CLSI breakpoints for M. catarrhalis, the non-susceptible rates of amoxicillin–clavulanic acid (>4 mg l⁻¹), levofloxacin (>2 mg l⁻¹), erythromycin (>2 mg l⁻¹), clarithromycin (>1 mg l⁻¹) and azithromycin (>0.125 mg l⁻¹) were 0% (0/47), 0% (0/47), 68.1% (32/47), 68.1% (32/47) and 68.1% (32/47), respectively.

**PFGE**

To investigate the possibility of clonal spreading of macrolide-resistant M. nonliquefaciens strains, 32 erythromycin-non-susceptible strains were evaluated by PFGE. The resulting dendrogram showed 30 PFGE patterns (Fig. 1). Strains in which a similar band pattern was detected (Mn8 and Mn10, as well as Mn13 and Mn19) had not been isolated from the same hospital.

**Analysis of macrolide-resistance targets**

In comparison with the 23S rRNA gene sequence (nt 1964–2430; Escherichia coli numbering, GenBank accession no.
V00331) of M. nonliquefaciens ATCC 17953 (GenBank accession no. AB818473), 18 different patterns of 23S rRNA gene sequences were identified in the 47 M. nonliquefaciens clinical isolates (GenBank accession nos AB818474–AB818489). Although a large number of mutations were observed in the 32 erythromycin-non-susceptible and 15 erythromycin-susceptible strains, mutations including A1986C, G1987A, A2058T, A2105G, T2113C, T2132A, A2150T, T2151C, G2154A, C2160T and T2184C, and an insertion at nt 2212 (E. coli numbering) were detected only in the erythromycin-non-susceptible strains. However, a single mutation, A2058T, was present only in erythromycin-non-susceptible strains and was found in all of these strains. The geometric mean MIC for erythromycin, clarithromycin, azithromycin and josamycin of 15 erythromycin-susceptible strains with the WT sequence at nt 2058 was significantly lower than those of 32 erythromycin-non-susceptible strains bearing the A2058T mutation (P<0.0001; Table 3).

Furthermore, five different amino acid sequence patterns in L4 and two in L22 were found among the 47 M. nonliquefaciens (data not shown). No sequence patterns present only in erythromycin-nonsusceptible strains were observed.

To investigate the presence of methylase genes, a PCR detection assay was performed. We did not detect ermA(A), ermA(B), ermA(C) or ermA(F) in any of the 47 clinical isolates.

Analysis of bro β-lactamase genes

Among the 47 M. nonliquefaciens clinical isolates, 43 (91.5%) were positive for bro-1 and 4 (8.5%) for bro-2. The results of DNA sequencing of bro-1 (165 bp) and bro-2 (144 bp) showed 100% similarity to those of M. catarrhalis (GenBank accession nos Z54180 and Z54181, respectively).

Analysis of the efflux system

MICs of erythromycin for the 47 M. nonliquefaciens isolates were evaluated in the absence or presence of efflux pump inhibitors. In the presence of PAbN, the MICs of 61.7% (29/47) isolates remained unchanged, 25.5% (12/47) were decreased twofold and 8.5% (4/47) were decreased fourfold (three erythromycin-non-susceptible strains: ⩽256 mg l⁻¹; one erythromycin-susceptible strain: 0.5 to ⩽0.125 mg l⁻¹) compared with the MICs in the absence of PAbN; 6.4% (3/47) did not grow. On the other hand, in the presence of CCCP, the MICs of 95.7% (45/47) remained similar to those observed in the absence of CCCP, while those of 6.4% (3/47) were decreased twofold.

DISCUSSION

To date, although there are few case reports of M. nonliquefaciens-associated infections, and M. nonliquefaciens has not yet been characterized in detail, including its antimicrobial susceptibility. In general, Moraxella species are susceptible to penicillin and its derivatives, cephalosporins, tetracyclines, quinolones, aminoglycosides, and macrolides (Vaneechoutte et al., 2012). Furthermore, previous studies have reported that M. nonliquefaciens is susceptible to penicillin G, ampicillin, ceftazidime, tetracycline, ciprofloxacin, gentamicin, erythromycin and imipenem (Davis et al., 2004; Laukeland et al., 2002). In this study, most M. nonliquefaciens demonstrated low penicillin MICs and showed extremely low MICs for ampicillin, amoxicillin, amoxicillin-sulbactam, amoxicillin-clavulanic acid, cefixime and levofloxacin, as reported in previous studies, while 68.1% (32/47) of clinical isolates demonstrated high-level MICs for 14-, 15- and 16-membered macrolides (e.g. erythromycin MIC >256 mg l⁻¹). Therefore, although the present study has certain
Deltically to the acquisition of resistance to macrolides, our mutation in the 23S rRNA gene may contribute synergis-
eritythromycin-susceptible strain. Therefore, although over-
eritythromycin-non-susceptible strains, but also in one
active efflux system was not only observed in three
strains did not share common amino acid sequences in
\( \text{erm}\_L4 \) and \( \text{L22} \), and
strains was significantly higher than that of WT strains
geometric mean MIC for macrolides in A2058T-mutated
erythromycin-non-susceptible strains. Furthermore, the
gene confers high-level resistance to macrolides and/or
mutation at position A2058 or A2059 in the 23S rRNA
\( \text{catarrhalis} \)
and
\( \text{M. pneumoniae} \)
recently, macrolide- and lincosamide-resistant \( \text{M. catar-
rhalis} \) strains have been reported in Japan (Saito et al.,
2012). Saito et al. (2012) demonstrated that the A2058T
mutation in the 23S rRNA allele (\( \text{E. coli} \) numbering) confers
high-level resistance to macrolides and lincosamides in \( \text{M. catarrhalis} \).
Furthermore, it has been described that a single
mutation at position A2058 or A2059 in the 23S rRNA
gene confers high-level resistance to macrolides and/or
lincosamides in a variety of bacteria, including \( \text{S. pneumoniae}, \text{Mycoplasma}
\text{smegmatis}, \text{Neisseria gonor-
rhoeae}, \text{Helicobacter pylori} \) and \( \text{M. pneumoniae} \) (Chironna et al.,
2011; Chisholm et al., 2010; Li et al., 2011; Tait-
Camradt et al., 2000; Versalovic et al., 1997). In the present
study, although 32 erythromycin-non-susceptible strains
harboured various sequencing profiles in the 23S rRNA
gene, a single A2058T mutation was common to all
erthyromycin-non-susceptible strains. Furthermore,
the geometric mean MIC for macrolides in A2058T-mutated
strains was significantly higher than that of WT strains
\( (P<0.0001) \). In contrast, erythromycin-non-susceptible
strains did not share common amino acid sequences in
L4 and L22, and \( \text{erm} \) genes were absent. Moreover, an
active efflux system was not only observed in three
erythromycin-non-susceptible strains, but also in one
erythromycin-susceptible strain. Therefore, although over-
expression of efflux systems in addition to the A2058T
mutation in the 23S rRNA gene may contribute synergis-
tically to the acquisition of resistance to macrolides, our
findings suggest the possibility that the presence of the
A2058T mutation is required to acquire high-level macro-
clide resistance in \( \text{M. nonliquefaciens} \), as described pre-
viously for \( \text{M. catarrhalis} \) (Liu et al., 2012; Saito et al.,
2012). However, as we have not investigated the role of
the A2058T mutation in the 23S rRNA allele of \( \text{M. nonliquefaciens} \) to the same extent as in our previous \( \text{M. catarrhalis} \) study (Saito et al., 2012), we propose to perform
further studies to analyse the role of the A2058T mutation
in more detail.

It is already known that \( \text{M. nonliquefaciens} \) produces BRO
\( \beta \)-lactamase (Eliasson et al., 1992). In this study, all 47 \( \text{M. nonliquefaciens} \) clinical isolates carried bro\(-\beta\)-lactamase
genes, and the frequency of bro-1- and bro-2-positive
isolates was 91.5 and 8.5 %, respectively. These observa-
tions are similar to previous findings of \( \text{M. catarrhalis} \),
which is closely related to \( \text{M. nonliquefaciens} \), in that more
than 90 % of the global clinical isolates are BRO \( \beta\)-
lactamase producers, of which bro-1-positive isolates
make up a large majority (Khan et al., 2010; Murphy &
Parameswaran, 2009).

The present study demonstrates that \( \text{M. nonliquefaciens} \) strains with high-level macrolide resistance have already
spread through clinical settings in Japan, but it is not clear
whether these results are related to the use of macrolides in
the treatment of infectious diseases, including respiratory
tract infections. Moreover, high-level macrolide-resistant
\( \text{M. catarrhalis} \) clinical isolates have already been reported in
Japan and China (Flamm et al., 2012; Liu et al., 2012; Saito
et al., 2012). To ensure rapid detection of the emergence
and prevention of further dissemination of high-level
macrolide-resistant phenotypes in \( \text{Moraxella} \) species, sur-
veillance of antimicrobial susceptibility to macrolides
should be continued.

In conclusion, we demonstrate that 68.1 % of \( \text{M. non-
liquefaciens} \) clinical isolates from Japan showed high-level
macrolide resistance and provide the first evidence that a
single A2058T mutation in the 23S rRNA gene confers this

Table 3. MICs of macrolides in WT and A2058T mutant strains of \( \text{M. nonliquefaciens} \)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>23S rRNA sequence( ^\text{a} )</th>
<th>( n )</th>
<th>MIC (mg l( ^{-1} ))</th>
<th>( P ) value( ^\text{b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>( \text{MIC}_{\text{so}} )</td>
<td>( \text{MIC}_{\text{so}} )</td>
<td>Geometric mean</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>WT</td>
<td>15</td>
<td>( \leq 0.125 )–1</td>
<td>( \leq 0.125 )</td>
</tr>
<tr>
<td></td>
<td>A2058T</td>
<td>32</td>
<td>512–&gt;512</td>
<td>512</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>WT</td>
<td>15</td>
<td>( \leq 0.125 )–0.5</td>
<td>( \leq 0.125 )</td>
</tr>
<tr>
<td></td>
<td>A2058T</td>
<td>32</td>
<td>128–&gt;512</td>
<td>512</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>WT</td>
<td>15</td>
<td>( \leq 0.125 )</td>
<td>( \leq 0.125 )–0.125</td>
</tr>
<tr>
<td></td>
<td>A2058T</td>
<td>32</td>
<td>64–&gt;512</td>
<td>256</td>
</tr>
<tr>
<td>Josa ycin</td>
<td>WT</td>
<td>15</td>
<td>0.5–4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>A2058T</td>
<td>32</td>
<td>128–256</td>
<td>256</td>
</tr>
</tbody>
</table>

\( ^{\text{a}} \text{E. coli numbering (GenBank accession no. V00331).} \)

\( ^{\text{b}} \text{Statistical significance determined by a Mann–Whitney }U\text{ test.} \)
high level of macrolide resistance. To our knowledge, this is the first report investigating the role of mutations in the 23S rRNA gene in \textit{M. nonliquefaciens}. As the emergence and spread of high-level macrolide-resistant \textit{Moraxella} species threatens effective treatment of these infections, continued surveillance for these strains should be undertaken.

**ACKNOWLEDGEMENTS**

We would like to express our gratitude to Professor Norihisa Noguchi who provided \textit{S. aureus} used as a positive control for \textit{erm(A)} and \textit{erm(C)}. The study did not receive financial support from third parties. The authors declare that they have no conflicts of interest.

**REFERENCES**


