Emergence and molecular characterization of Haemophilus influenzae harbouring mef(A)

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Sir,

Haemophilus influenzae is an indigenous bacterium of the upper respiratory tract and, along with Streptococcus pneumoniae, can cause respiratory tract infections. Although β-lactams are generally used for the treatment of these infections, β-lactamase-producing ampicillin-resistant H. influenzae and β-lactamase-non-producing ampicillin-resistant H. influenzae strains are well documented.1 Furthermore, isolates with reduced susceptibility to macrolides, which can be used as alternative agents, have also been reported.2,3 There are several known mechanisms of macrolide resistance in H. influenzae,2,4 which involve mutations in chromosomal genes rather than in acquired genes. However, in 2011, the acquisition of resistance genes such as mef(A), which encodes a drug efflux pump, and erm(A), erm(B), erm(C) and erm(F), which encode 23S rRNA methylases, was reported in H. influenzae isolated from patients with cystic fibrosis.5 Nonetheless, this study is currently the only report of these acquired macrolide resistance genes in H. influenzae.5 Although Atkinson et al.5 found no clear association between the presence of these genes and the MICs of macrolides, and reported that these genes were not widespread in clinical isolates of H. influenzae in 2015, the primer sets used by Atkinson et al. were not appropriate. Therefore, the existence and prevalence of acquired macrolide resistance genes in H. influenzae remain to be determined.

We isolated non-typeable H. influenzae strain 2014-102 from the sputum of a paediatric patient with respiratory infection at the Tokyo Medical University Hachioji Medical Center in 2014, which showed high-level resistance to macrolides (azithromycin MIC = 64 mg/L; clarithromycin MIC = 128 mg/L). In the present study, we analysed the genes in this isolate and characterized their contribution to macrolide resistance.

H. influenzae 2014-102 was identified as ST478 by MLST. Based on a previous study,2 the MICs of macrolides were determined in the presence of the efflux pump inhibitor CCCP. The MICs of both azithromycin and clarithromycin decreased in the presence of CCCP (Table S1, available as Supplementary data at JAC Online). However, no overexpression of the chromosomal multidrug efflux pump acrAB was observed in an RT-PCR assay.6 In addition, no amino acid substitutions were detected in either the L4 or L22 ribosomal protein, and no mutations were detected in 23S rRNA. Conversely, PCR revealed the presence of the macrolide resistance gene mef(A), but not erm(B). To confirm whether the DNA detected by PCR was indeed mef(A), the amplified DNA was sequenced and analysed. The sequence of mef(A) carried by H. influenzae 2014-102 showed 100% homology to mef(A) reported in streptococci. Consequently, we concluded that mef(A) is most likely involved in the macrolide resistance of H. influenzae 2014-102.

Furthermore, sequencing analysis of the flanking region indicated that tet(M), which encodes ribosome protection proteins, was located close (3309 bp) to mef(A). The tet(M) gene also showed 100% homology to that of streptococci. The region (6445 bp) from tet(M) to mef(A) in H. influenzae 2014-102 was further investigated. A BLAST search of this region showed that it was identical to part of the Tn916 family transposon (~20 kb) of S. pneumoniae ST556 (GenBank accession no. CP003357) and S. pneumoniae Taiwan 19F-14 (GenBank accession no. CP000921) (Figure 1).

H. influenzae 2014-102 showed resistance to penicillin and minocycline, in addition to macrolides (Table S1). Penicillin resistance was mediated by β-lactamase blaTEM-1, but not by a mutation in PBP3. Certain resistance genes, including mef(A), have been demonstrated to transfer among S. pneumoniae.7 H. influenzae also has a transforming ability similar to streptococci.6,8 Both species inhabit the same sites. These results indicate that H. influenzae 2014-102 obtained mef(A) and tet(M) from other species via horizontal gene transfer. To test whether these resistance genes were transferable via mobile genetic elements, we performed conjugation experiments using H. influenzae Rd, H. influenzae clinical isolates and S. pneumoniae clinical isolates. However, there was no evidence that resistance genes were transferred (data not shown).

H. influenzae harbouring the tet(M) gene alone has been reported previously, but mef(A)-harbouring H. influenzae has not been identified.5,6,10 This is the first report of the acquisition of both the mef(A) and tet(M) genes in H. influenzae. Nevertheless, these acquired macrolide resistance genes are not widespread, as this is the only strain carrying mef(A) among 449 isolates investigated from 2007 to 2014.10 The prevalence of macrolide resistance genes might be dependent on the population and their level of exposure to macrolides.

Our data also strongly suggested that the mef(A) and tet(M) genes likely transfer at the same time. Therefore, it is necessary to monitor carefully the potential expansion of resistant strains that acquire both of these resistance genes.

Nucleotide sequence accession number
The analysed nucleotide sequence was submitted to DDBJ/NCBI/GenBank under accession no. LC168847.
S. pneumoniae (accession no. CP003357)

1 Ubukata K, Shibasaki Y, Yamamoto K. References. journals.org/).

Table S1 is available as Supplementary data at Supplementary data.


Figure 1. Analysis of flanking regions of the tet(M) and mef(A) genes. The area between two lines shows a gene with high homology. H. influenzae 2014–102, mef(A)- and tet(M)-positive strain; S. pneumoniae ST556, reference sequence (accession no. CP003357). MYY_1868 and CDS_1, hypothetical protein; MYY_1869 and CDS_2, ImsC-like protein; MYY_1870 and CDS_3, hypothetical protein; MYY_1871 and CDS_4, hypothetical protein; MYY_1872 and CDS_5, ABC transporter msr(D).

**Supplementary data**

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**


**Evidence of transmission of an NDM-5-producing Klebsiella pneumoniae in a healthcare facility in New Zealand**

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Sir,

Carbapenemase-producing Enterobacteriaceae (CPE) are still relatively rarely isolated in New Zealand (NZ). Between 2009 and 2014, only 35 confirmed, non-duplicate isolates were reported to the national CPE surveillance system.1 However, the prevalence is increasing, with 41 isolates reported in 2015.2 In NZ to date, the majority of patients with CPE have had a history of hospitalization or travel within countries with a high prevalence of CPE. However, for the first time in 2015, transmission of CPE in NZ healthcare facilities was identified.2 There were two such events, one of which was in our hospital. Here, we describe that event, which involved four patients with the same strain of Klebsiella pneumoniae with blaNDM-5.

The first isolate was identified in a faecal sample, sent from a haematology patient for routine screening for MDR organisms, ESBL, CPE, MRSA and VRE, in September 2015. Our hospital infection policy states that haematology inpatients are screened on admission for ESBL, CPE, VRE and MRSA and subsequently on a