Occurrence and diversity of the tetracycline resistance gene tet(M) in enteric bacteria of Antarctic Adélie penguins

M. Habibur Rahman1, Kentaro Q. Sakamoto2, Lisa Nonaka1 and Satoru Suzuki1*

1Centre for Marine Environmental Studies (CMES), Ehime University, Matsuyama 790-8577, Japan; 2Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Keywords: Antarctica, marine ecosystem, drug resistance, ribosomal protection protein, selective pressure

The tet(M) gene has been found in numerous bacterial genera isolated from various environments.1 However, there is no report on the investigation of the tet(M) gene in Antarctica. We investigated the occurrence of tet(M) in the isolates from Antarctic Adélie penguins (Pygoscelis adeliae) and detected two different genotypes of tet(M).2

Sampling was carried out from Adélie penguins colonizing Hukuro Cove (69°13’ S, 039°39’ E), Lützow-Holm Bay, Antarctica, from December 2004 to February 2005, as an activity of the 45th Japanese Antarctic Research Expedition (JARE45). Intestinal contents (faeces) were collected by insertion of a sterilized cotton swab into the cloaca. Viable bacterial count was performed on marine broth 2216E (Difco) plus 1.5% BactoTM agar containing 0 or 30 mg/L tetracycline. The plates were incubated at 25°C for 7 days, and colonies were counted. Chromosomal DNA was extracted from 23 tetracycline-resistant (TeC) isolates. Isolates were classified by 16S rDNA analysis. Specific primers for tet(M) detection, tet(M)-1 (5'-GTAAATAGTTCTTGGAG-3') and tet(M)-2 (5'-CTAAGATGCTCTAACAA-3'), were used for PCR; the product length was expected to be 657 bp.3 The PCR products were cloned and sequenced.

The TeC bacteria represented 0% to 0.54% of the total viable count. The TeC bacteria included isolates from the following genera: Bacillus, Brachybacterium, Streptomyces, Arthrobacter, Psychrobacter and Pseudomonas (Table 1). Currently, 40 TeC genes have been reported (http://faculty.washington.edu/marilynnr/). Among these, the tet(M) gene has been detected in a wide range of bacterial species and is the most widely distributed. Thus, carriage of the tet(M) gene was determined. Of 23 isolates, 10 were TeC and carried the tet(M) gene, and included 10 Bacillus spp., 4 Brachybacterium spp., 2 Streptomyces spp. and 1 Pseudomonas sp. (Table 1). To our knowledge, this is the first study on the detection of tet(M) in an animal endemic to Antarctica. This study is the first description of acquired tet genes tet(M) in either Brachybacterium or Arthrobacter. We also suggest that Antarctic Adélie penguins may act as a reservoir for TeC bacteria because they have the potential to spread these TeC isolates.

Table 1. The presence of the tet(M)-positive bacteria among 23 TeC isolates

<table>
<thead>
<tr>
<th>Classification</th>
<th>(by 16S rDNA analysis)</th>
<th>tet(M)-positive isolates/total TeC isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td></td>
<td>3/10</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachybacterium spp.a</td>
<td></td>
<td>4/4</td>
</tr>
<tr>
<td>Streptomyces spp.</td>
<td></td>
<td>2/2</td>
</tr>
<tr>
<td>Arthrobacter spp.a</td>
<td></td>
<td>1/1</td>
</tr>
<tr>
<td>Gamma-proteobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychrobacter spp.</td>
<td></td>
<td>0/5</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td></td>
<td>0/1</td>
</tr>
</tbody>
</table>

*aFirst tet(M)-positive isolate from this genus.
bacteria to their young through regurgitation feeding and throughout the Antarctic ecosystem via faecal contamination.

Acknowledgements

We thank Dr Todd Miller for his critical review of the manuscript.

Funding

This study was partly supported by Grants-in-Aid from the 21st Century-COE and Global-COE Programmes (MEXT) and Grants-in-Aid from JSPS (14208063).

Transparency declarations

None to declare.

References


Journal of Antimicrobial Chemotherapy

doi:10.1093/jac/dkn202
Advance Access publication 12 May 2008

Macrolide resistance among invasive Streptococcus pneumoniae in Slovenia

Tamara Kastrin1*, Marija Gubina2, Metka Paragi1, Jana Kolman3, Milan Čižman4, Alenka Kraigher1, Helena Ribič5, Ewa Sadowy6 and Waleria Hryniewicz6

1Department of Medical Microbiology, Institute of Public Health of the Republic of Slovenia, Grabloviceva 44, 1000 Ljubljana, Slovenia; 2School of Health Science, Higher Education Centre Novo mesto, Na Loko 2, 8000 Novo mesto, Slovenia; 3University Medical Centre Ljubljana, Infection Control Service, Japljeva 2, 1525 Ljubljana, Slovenia; 4Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, 1525 Ljubljana, Slovenia; 5The Institute of Public Health Kranj, Gosposvetska 12, 4000 Kranj, Slovenia; 6National Medicines Institute, Chelmska 30/34, 00-725 Warsaw, Poland

Keywords: pneumococci, erythromycin, serotype, erm(B), mef(A)

*Corresponding author. Tel: +386-1-5205-708; Fax: +386-1-5205-704; E-mail: tamara.kastrin@ivz-rs.si

Sir,

The worldwide increase in antibiotic resistance of Streptococcus pneumoniae has become a serious problem in recent years, including the increasing resistance to macrolides. In Slovenia, the epidemiological situation of invasive diseases, caused by S. pneumoniae, has been constantly monitored since 1993.1,2

The objectives of this study were to characterize the macrolide-resistant clinical isolates of invasive S. pneumoniae in Slovenia and to investigate the genetic basis of macrolide resistance.

A total of 1448 invasive isolates of S. pneumoniae, recovered from blood cultures and from cerebrospinal fluid, were collected in Slovenia from 1998 to 2007. Of these, 399 isolates (27.6%) were obtained from children (0–14 years of age) and 1049 (72.4%) from adult patients. The isolates were serotyped by Neufeld’s Quellung reaction using antisera provided by the Statens Serum Institut, Copenhagen, Denmark. Antibiotic susceptibility was determined following the recommendations of the CLSI.3 The presence of erm(A), erm(B) and mef(A) genes were determined by PCR with primers and reaction conditions according to Sutcliffe et al.4 Isolates negative for the acquired macrolide genes had their domain V and II from all four 23S rRNA alleles and the genes coding for ribosomal proteins L4 and L22 sequenced.5

A rise of macrolide resistance (MIC ≥ 1 mg/L) in invasive S. pneumoniae from 4.7% in 1998 to 16.8% in 2007 was observed in Slovenia. The rate of resistance increased 5.7-fold in the case of isolates from children, from 4.3% in 1998 to 24.6% in 2007 (Table 1). At the same time, however, the consumption of macrolides in Slovenia decreased by 36% from 1999 to 2007 (from 3.81 to 2.43 defined daily doses per 1000 inhabitants per day), which had no influence on the increase in resistance.

Fifty-nine percent of the macrolide-resistant pneumococci showed the constitutive MLSB phenotype and 41% the M phenotype. No erythromycin–clindamycin inducible resistant strains were found.

The most prevalent determinant of macrolide resistance was the erm(B) gene, which was present in 86 isolates (55%), followed by the mef(A) gene, which was present in 61 isolates (39%). The erm(A) gene was not found.

The erythromycin MICs for the isolates carrying the erm(B) gene were ≥256 mg/L and for the isolates with the mef(A) gene, they ranged between 2 and 32 mg/L, except for a single isolate with MIC ≥ 256 mg/L. In this isolate, we also found a mutation in the rrl gene coding for 23S rRNA, A2059G in all four alleles. Three other isolates also harboured mutations in the rrl gene; we demonstrated the A2059G substitution in three of four alleles, A2059G in all four alleles and A2058C in two of four alleles (Escherichia coli numbering). They had the MLSB phenotype and erythromycin MICs ≥ 256 mg/L. In one isolate also with MIC ≥256 mg/L, we determined a mutation in the rplV gene, coding for ribosomal protein L22. It had a 6-amino-acid duplication (103RTAHIT). All the mutations identified have previously been described.5 The change Ser20-Asn in L4 ribosomal protein, which was found in two isolates, has already been described and it does not confer resistance.6 In three isolates, the mechanism of resistance could not be established.

The acquisition of a resistance determinant often provides a selective advantage for a pneumococcal strain and promotes its clonal spread. It is not surprising therefore that, among