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Plasmid-mediated quinolone resistance determinants in Salmonella spp.
isolates from reptiles in Germany

Beatriz Guerra*, Reiner Helmuth, Katharina Thomas, Janine Beutlich, Silke Jahn and Andreas Schroeter

Federal Institute for Risk Assessment, BfR, Department for Biological Safety, Diederstorfer Weg 1, D-12277 Berlin, Germany

*Corresponding author. Tel: +49-30-8412-2082; Fax: +49-30-8412-2953; E-mail: beatriz.guerra@bfr.bund.de

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Sir,

In 2008, the European Community Reference Laboratory for Antimicrobial Resistance (EURL-AR; at the National Food Institute, Denmark) asked the European National Reference Laboratories within their network (NRLs-AR) to collect retrospective information on the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants in Salmonella and Escherichia coli isolates. In Germany, we screened the data of >40,000 Salmonella strains (NRL-Salm collection) isolated in all Federal Länder of Germany, originating from routine surveys of different investigation centres involved in public health, or from national or EU monitoring programmes obtained during 2000–08. These isolates originated from food-producing animals (39%), foods (28%), non-food-producing animals (14%) [including 1900 isolates (5%) from reptiles], animal feed (8%), environments (7%), humans (3%) and other sources (2%). Among these isolates, 194 showed database records of MICs (determined by the CLSI broth microdilution method) suggesting the presence of PMQR-encoding genes (nalidixic acid MICs of 8–32 mg/L and ciprofloxacin MICs of 0.06–1 mg/L). However, in secondary tests (Etest and CLSI agar dilution) performed in June 2009 for all these isolates, only for 113 could the initial MIC values be confirmed. Interestingly, 14 of them (12.4%) originated from reptiles. Avoiding duplicated isolates (same isolation place/date), 10 of these reptile isolates were selected for this study.

The 10 isolates showed nalidixic acid MICs of 8–32 mg/L and ciprofloxacin MICs of 0.25–1 mg/L. They were tested for the presence of the PMQR-encoding genes qnrA, qnrB, qnrC, qnrD, qnrS, qepA and aac(6’)-Ib-cr by PCR amplifications/sequencing using the primers and conditions described in Table S1 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org)]. To determine epidemiological relationships, the isolates were typed by PFGE with XbaI (Roche Diagnostics, Germany) using the PulseNet protocol (www.pulsenetinternational.org) [Figure S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org)]. Plasmid profile analysis was done by DNA extraction followed by separation on 0.8% vertical agarose gels (Figure S1). Plasmid DNA was transferred via Southern blotting and hybridized with probes for the different PMQR genes detected (Figure S1). The incompatibility group of the plasmids was determined by a PCR replicon-typing scheme.

Nine of the 10 isolates were positive for some of the PMQR genes cited above. In one isolate, none of these genes was present. The qnrB19 gene was present in seven strains belonging to different Salmonella subspecies and serotypes, isolated from different reptiles in several German regions (Table 1). The two Salmonella Urbana and one of the rough isolates were isolated from different animals in the same zoo during different isolation periods, and showed very similar XbaI-PFGE and plasmid patterns (Figure S1), suggesting the clonal spread of a qnrB19-positive strain. The qnrS1 gene, and qnrB6 together with aac(6’)-Ib-cr, were found in isolates from turtles. No qnrA, qnrC, qnrD or qepA genes were detected.

Plasmid location of the qnr genes was confirmed (Figure S1). The qnrB19 gene was carried on plasmids of ~4.5 kb (four isolates) and ~3 kb (three isolates). All these plasmids belonged to the incompatibility group CoIE1, representing the CoE-Tp variant described for small plasmids (~10 kb) isolated from different Salmonella serotypes. Very small qnrB19 plasmids (2.7 kb) belonging to this incompatibility group have recently been described. The present study shows that these plasmids seem to be more frequent than previously observed.

The qnrB6 gene was located on an ~45 kb plasmid belonging to IncN and qnrS1 was located on an ~35 kb IncX plasmid (Figure S1). qnr genes have been found on plasmids belonging to several incompatibility groups, being frequent in Inc F, N, L/M and CoE plasmids, but as far as we know they have not been described on IncX plasmids, a family commonly found in Salmonella.

Our results show that various determinants conferring PMQR are present in Salmonella isolates originating from different reptile species. This has also been found in different bacteria (i.e. Pseudomonas, Klebsiella and E. coli) isolated from zoo reptiles (four isolates from tigers being positive for qnrB and qnrS1) in Japan, but we could not find in the published literature any other description of the presence of PMQR in Salmonella isolated from reptiles.

We do not know if the presence of PMQR could be related to the treatment of zoo or pet animals, or if they are colonized by resistant isolates via feed. Although the Salmonella serovars described rarely appear in human disease in Germany (i.e. since 2001, there have been 2–6 cases per year of Salmonella Urbana in humans; Robert Koch Institute, www3.rki.de/SurvStat), some of them, such as Salmonella Urbana, are highly invasive and infected patients might require treatment with fluoroquinolones. Furthermore, these animals could serve as a reservoir for the further spread of these determinants. For
<table>
<thead>
<tr>
<th>NRL no.</th>
<th>Serotype</th>
<th>Origin</th>
<th>Isolation region (city)</th>
<th>MIC (mg/L)</th>
<th>XbaI PFGE pattern</th>
<th>Plasmid profile</th>
<th>PMQR genes</th>
<th>Carrier plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>07-1678</td>
<td>Salmonella Urbana</td>
<td>black iguana (zoo)</td>
<td>Berlin</td>
<td>susceptible</td>
<td>0.5</td>
<td>16</td>
<td>X1</td>
<td>PP1</td>
</tr>
<tr>
<td>08-0944</td>
<td>Salmonella subsp. I</td>
<td>water dragon (zoo)</td>
<td>Berlin</td>
<td>susceptible</td>
<td>0.5</td>
<td>16</td>
<td>X1</td>
<td>PP1</td>
</tr>
<tr>
<td>07-4392</td>
<td>Salmonella Urbana</td>
<td>bearded dragon (zoo)</td>
<td>Berlin</td>
<td>susceptible</td>
<td>0.25</td>
<td>8</td>
<td>X1b</td>
<td>PP1b</td>
</tr>
<tr>
<td>08-4326</td>
<td>Salmonella subsp. I</td>
<td>bearded dragon</td>
<td>Baden-Württemberg (Stuttgart)</td>
<td>susceptible</td>
<td>0.5</td>
<td>16</td>
<td>X2</td>
<td>PP2</td>
</tr>
<tr>
<td>06-1428</td>
<td>Salmonella subsp. II</td>
<td>gecko</td>
<td>Brandenburg (Frankfurt an der Oder)</td>
<td>SXT–NAL</td>
<td>1</td>
<td>32</td>
<td>X3</td>
<td>PP3</td>
</tr>
<tr>
<td>05-4864</td>
<td>Salmonella subsp. IV</td>
<td>monitor lizard</td>
<td>Thuringia (Jena)</td>
<td>susceptible</td>
<td>0.5</td>
<td>16</td>
<td>X4</td>
<td>PP4</td>
</tr>
<tr>
<td>06-0145</td>
<td>Salmonella subsp. IV</td>
<td>snake</td>
<td>North Rhine-Westphalia (Detmold)</td>
<td>susceptible</td>
<td>0.5</td>
<td>16</td>
<td>X5</td>
<td>PP5</td>
</tr>
<tr>
<td>07-0279</td>
<td>Salmonella Litchfield</td>
<td>turtle</td>
<td>Thuringia (Bad Langensalza)</td>
<td>SXT–TET–TMP</td>
<td>1</td>
<td>16</td>
<td>X6</td>
<td>PP6</td>
</tr>
<tr>
<td>08-4339</td>
<td>Salmonella Pomona</td>
<td>turtle</td>
<td>Hesse (Gießen)</td>
<td>TET–TMP</td>
<td>0.5</td>
<td>8</td>
<td>X7</td>
<td>PP7</td>
</tr>
</tbody>
</table>

NRL, German National Salmonella Reference Laboratory (NRL-Salm).
<sup>a</sup>European Committee on Antimicrobial Susceptibility Testing (www.eucast.org) cut-off values for microdilution susceptibility testing were used. The antimicrobials tested were: NAL, nalidixic acid; TET, tetracycline; TMP, trimethoprim; SXT, trimethoprim/sulfamethoxazole.
<sup>b</sup>After molecular analyses (PFGE typing), this isolate was considered as Salmonella Urbana.
<sup>c</sup>Only the qnrB probe was used for hybridization experiments.
<sup>ColE variant described by Garcia-Fernández et al. 3</sup>

Table 1. Salmonella enterica carrying PMQR genes isolated from reptiles
all these reasons, the presence of PMQR in reptiles should be seen as a public health concern.

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Transparency declarations

None to declare.

Supplementary data

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

References


Long-term stability of temocillin in elastomeric pumps for outpatient antibiotic therapy in cystic fibrosis patients

Stéphane Caryyn1,2†, Nathalie Couwenbergh1 and Paul M. Tulkens1*

1. Unité de pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, B-1200 Bruxelles, Belgium; 2. Eumedica S.A., B-7170 Manage, Belgium

*Corresponding author. Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, UCL7370 avenue E. Mounier 73, B-1200 Bruxelles, Belgium. Tel: +32-2-7647236; Fax: +32-2-7647373; E-mail: tulkens@facm.ucl.ac.be
†Present address: GlaxoSmithKline Biologicals S.A., B-1300 Wavre, Belgium.

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Sir,

Outpatient antibiotic therapy (OPAT) is often proposed to cystic fibrosis (CF) patients in order to reduce the risk of cross-infection and the duration of hospital stay.1 However, the long-term stability of the drug in the home-supplied device needs to be assessed under conditions mimicking their projected use before any large-scale implementation of OPAT. This is particularly critical for β-lactam antibiotics, as these are notoriously unstable in aqueous solutions; however, there are quite large variations among them.2 We previously reported that temocillin, a 6α-methoxy-carboxypenicillin with exceptional stability in the presence of most β-lactamases, including extended-spectrum β-lactamases,3 is very stable even when kept at 37°C for several hours in concentrated solutions, as required for use in continuous infusion.4 While being useless for infections caused by Pseudomonas aeruginosa or Acinetobacter spp., temocillin shows good in vitro activity against Burkholderia cepacia complex (Bcc),5 a difficult-to-treat opportunistic organism that often affects vulnerable individuals such as CF patients.6 Since most Bcc isolates are resistant to many, if not all, other antibacterial agents commonly used in CF patients, temocillin could be potentially useful, and even life-saving, in severe pulmonary exacerbations.

We have assessed the stability of temocillin in two frequently used elastomeric devices, namely: (i) the Easypump® 100-0.5 (1-Flow Corp., Lake Forest, CA, USA; also called Homepump Eclipse® in some other countries); and (ii) the Interma-te® SV200 (Baxter Healthcare Corp., Deerfield, IL, USA), taking care to mimic the actual projected use of these devices for OPAT in CF patients. Thus, 30 pumps from each brand were loaded at room temperature with concentrated temocillin

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