Comment on: Acquired macrolide resistance genes in Haemophilus influenzae?

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Sir,

We read the recently published paper of Atkinson et al.1 with interest. The authors have misrepresented and misquoted our study 2 by referencing primers we did not use or advocate [Table 1 and Table S1 (available as Supplementary data at JAC Online)].1 The erm(A) and mef(A) primer sets Atkinson et al.1 reported to have produced false-positive PCR results were not used in our study nor described in our paper (Table 1).2 The erm(A) primers they attributed to our study were degenerate primers that have never been used by our group.2 – 7 We found that the primers for erm(A), erm(B) and erm(F) genes listed in Atkinson et al.1 (Table S1) were actually designed and reported previously by Chen et al.8 in a study on the use of rapid real-time PCR assays for the quantification of erm genes in manure, compost, lagoons and a bioreactor. Clearly, when used for Haemophilus influenzae they gave false positives and another primer set should have been used for detection of the genes in question. The erm(A) and mef(A) primer sets used for our H. influenzae study (Table 1),2 and that do not produce false-positive PCR results, have been described previously.3 – 7

In addition to confirming our positive PCR results by hybridization of the PCR products with internal probes, we verified that the macrolide genes are on mobile elements by selecting 25 H. influenzae isolates as donors for mating experiments with H. influenzae Rd and/or Enterococcus faecalis JH2-2 as recipients.2 All 25 donors were able to transfer macrolide resistance to recipients at a frequency ranging from 10−7 to 10−10/recipient.2 The resulting transconjugants were resistant to macrolides and had erm genes and/or a mef(A) gene that were originally found in the donor strains.2 Mutations do not normally transfer by conjugation (M. C. Roberts, unpublished observations).

We should emphasize that a study of 172 H. influenzae isolates from Australia should not be considered representative of H. influenzae across the world. A possible reason for differences between the USA and Australian isolates may be the type of patients included in the Australian study.1 Only 15.7% (27/172) of the Australian H. influenzae isolates came from patients with exposure to azithromycin compared with 106 USA cystic fibrosis isolates that were from patients participating in a clinical study where 50% had extensive azithromycin therapy three times per week.2 Only 7 Australian isolates were macrolide resistant compared with 27 macrolide-resistant isolates in the USA study.1,2 Therefore, based on different populations (azithromycin exposed) and a low number of isolates resistant to macrolides, there could easily be differences in the distribution of erm/mef(A) genes among H. influenzae from different patient populations even within a single city.

It would have been valuable had Atkinson et al.1 used PCR-specific primers with PCR assays and not with real-time PCR.

Table 1. Forward, reverse and internal primers for macrolide resistance genes used in the Roberts et al.2 H. influenzae study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Internal</th>
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<tbody>
<tr>
<td>erm(A)</td>
<td>ERM AF: TCT AAA AAG CAT GTA AAA GAA</td>
<td>ERM AR: AGT AAC GGT ACT TAA ATT TAC</td>
<td>ERM A-INT: CCT GAT CCG ATT TCT ATT ACG TTG</td>
</tr>
<tr>
<td>erm(B)</td>
<td>ERM BF: GAA AAG GTA CTC AAC CAA ATA</td>
<td>ERM BR: AGT AAC GGT ACT TAA ATT TAC</td>
<td>ERM B-INT: AGC CAT GCG TCT GAC ATC TAT</td>
</tr>
<tr>
<td>erm(C)</td>
<td>ERM CF: TCA AAA CAT AAT ATA GAT AAA</td>
<td>ERM CR: GCT AAT ATT TAA ATT TAC</td>
<td>ERM C-INT: CGT GGA ATA CCG GTT TGC TA</td>
</tr>
<tr>
<td>erm(F)</td>
<td>ERM F1: CGG GTC AGC ACT TTA CTA TTG</td>
<td>ERM F2R: GGA CCT ACC TCA TAG ACA AG</td>
<td>ERM F-INT: TGA TGC CCG AAA TGT TCA AGT</td>
</tr>
<tr>
<td>mef(A)</td>
<td>MEF IN FW: TGT GCA TAT TTC TAT TAC</td>
<td>MEF IN RV: CCA ATT GGC ATA GCA AG</td>
<td>MEFI: GCT GTGCAA TAA TGG GGC</td>
</tr>
</tbody>
</table>

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assays. Without this being done, there is no scientific evidence for the authors to conclude that ‘It is interesting to speculate as to whether some of the positive results in the Roberts et al. study, particularly for mef(A) and erm(A), could have been similar false positives’, especially since our strains were internal probe positive and the 25 isolates used for mating experiments could move macrolide resistance by conjugation. It would have been interesting if the resistant Australian strains were tested on their ability to transfer macrolide resistance to a recipient, which would have indicated the presence of a known or unidentified/novel acquired macrolide resistance gene. Our group and collaborators from Chile and Germany have reported previously that it is possible to transfer tetracycline resistance from Chilean salmon farm isolates (Pseudomonas putida and Providencia rettgeri) with unknown tet genes to recipient Escherichia coli strains HB101 and DH5α.

One last note: there is no mef(E) gene, it is just a variant of mef(A) with >80% amino acid identity to mef(A) and it is not recognized as a separate gene.

Transparency declarations
None to declare.

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

References

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Acquired macrolide resistance genes in Haemophilus influenzae?—authors’ response

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Sir,

In reply to the letter ‘Comment on: Acquired macrolide resistance genes in Haemophilus influenzae?’ by Roberts et al., where the authors provide commentary on our respective publications,2,3 we offer the following response.

The paper by Roberts et al.2 was remarkable in that at least one acquired macrolide resistance gene was found in all 106 isolates of H. influenzae tested, and, because of our long-standing interest in antimicrobial resistance in H. influenzae, we were interested in determining whether the presence of these genes was more widespread. We chose to design and use highly specific locked nucleic acid hydrolysis probe-based PCR on our collection of 172 isolates and did not detect any of the macrolide resistance genes reported by Roberts et al.2,3

For reasons we describe in our paper,3 we attempted to retest our isolates using the primers from the Roberts et al.1 study, but we had some difficulty doing so. It is important to note that the table of primer sequences (Table 1, Roberts et al.1) that accompanies the letter by Roberts et al.1 was not included in the original publication, and, further, the references in that table were not cited in reference to PCR protocols in the original publication. Instead, in the Methods section of their study,2 they report that the erm(B), erm(F), msr(A) and mef(A) genes were tested for ‘as previously described’ in Soge et al.,4 Soge et al.5 and Ross et al.,6 and the erm(A) and erm(C) genes were tested for ‘as previously described’ in Soge et al.,4,6 but these references do not directly or adequately describe the primers or the associated protocols. Ross et al.2 refer only to msr(A), which was not further discussed by Roberts et al.2 and was not included in our study. Soge et al.5 describe neither primers nor conditions for PCR detection of macrolide resistance genes, but they refer the reader to Luna et al.1 for details on erm(A), erm(B) and erm(C) PCR. In Soge et al.1, erm(A), erm(C) and erm(F) primers are not described, although erm(B) primers are clearly described (and correspond to those for erm(B) in Table 1), and the primers MEFF and MEFR

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