Conjugative transfer frequencies of \textit{mef}(A)-containing \textit{Tn}1207.3 to macrolide-susceptible \textit{Streptococcus pyogenes} belonging to different \textit{emm} types

N.F. Hadjirin\(^1\), E.M. Harrison\(^2\), M.A. Holmes\(^2\) and G.K. Paterson\(^2\)

\(^1\) University of West London, London, UK
\(^2\) Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

Significance and Impact of the Study: The spread of antimicrobial resistance among pathogenic bacteria is an important problem, but the mechanisms of horizontal transfer between strains and species are often poorly understood. For instance, little is known on how macrolide resistance spreads between strains of the human pathogen \textit{Strep. pyogenes} and why certain strains more commonly display resistance than others. Here, we show that \textit{Strep. pyogenes} strains vary greatly in their ability to acquire a transposon encoding macrolide resistance by horizontal gene transfer \textit{in vitro}. These data provide a novel insight into the transfer of antibiotic resistance between bacterial strains and offer an explanation for the differences in the frequency of resistance determinates and resistance seen among clinical isolates.

Keywords
antibiotics, molecular epidemiology, streptococci, transposons.

Correspondence
Nazreen F. Hadjirin and Gavin K. Paterson, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK. E-mails: nh396@cam.ac.uk and gkp27@cam.ac.uk

Present address
N.F. Hadjirin, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, UK

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Abstract
The aim of this study was to examine the gene transfer potential of \textit{mef}(A)-containing \textit{Tn}1207.3 to macrolide-susceptible \textit{Streptococcus pyogenes} belonging to different \textit{emm} types. Using the filter mating technique, \textit{Tn}1207.3 was transferred by conjugation to 23 macrolide-susceptible recipients representing 11 \textit{emm} types. PCR analysis confirmed the presence of the \textit{mef}(A) gene and the \textit{comEC} junction regions of the \textit{Tn}1207.3 insertion in resultant transconjugants. Significant variation was found in the transfer frequency of \textit{Tn}1207.3 to different \textit{Strep. pyogenes} strains, and this phenomenon may contribute to the differences in \textit{mef}(A) frequency observed among clinical isolates.

Introduction
\textit{Streptococcus pyogenes} (group A streptococci/GAS) remains an important human pathogen responsible for a wide variety of invasive and noninvasive infections including pharyngotonsillitis, skin and soft tissue infections and bacteraemia. Macrolides are widely used to treat GAS infections in patients allergic to \textit{\beta}-lactam antibiotics.

The development and spread of macrolide resistance among GAS throughout the world is therefore a major concern.

The drug efflux pump, encoded by the \textit{mef} gene (currently represented by four subclasses A, E, I and O), is one of the most common mechanisms responsible for resistance to 14- and 15-membered macrolides in GAS and other species (Sutcliffe \textit{et al.} 1996a; Varaldo \textit{et al.} 1996b; Menon \textit{et al.} 1997).
2009). At least three mobile elements carrying the mef(A) gene have been identified in GAS: the Tn1207.3 transposon (Santagati et al. 2003), the Φ10394.4 phage chimera (Banks et al. 2003) and the 9m46.1 element (Brenciani et al. 2010). Studies indicate a significant prevalence of the mef(A) gene among GAS isolates in Europe and elsewhere. For instance, nationwide surveillance in Germany noted a frequency of 31% (Bley et al. 2011), while an Italian study found the prevalence of mef(A) among GAS to be 16% (Creti et al. 2005). Interestingly, there appears to be a skewed distribution of macrolide resistance genes among different GAS strains belonging to different emm types. The mef(A) gene is most frequently harboured by certain emm types such as emm1, emm2, emm3, emm4, emm9, emm12 and emm75, whereas emm types such as emm22, emm77, emm87 and emm89 have not yet been documented to carry any mef determinant (Creti et al. 2005; Grivea et al. 2006; Silva-Costa et al. 2008; Wajima et al. 2013). These observations suggest differences in the capacity of GAS emm types to acquire mef genes, and while the horizontal gene transfer of mef(A)-bearing genetic elements has been demonstrated in vitro by conjugation (Giovanetti et al. 2003; Santagati et al. 2003) and by transduction (Di Luca et al. 2010), no comparison has yet been made of mef gene transfer frequencies among different GAS strains.

The aim of this study therefore was to examine the transfer frequency of mef(A)-containing Tn1207.3 to diverse macrolide-susceptible recipients representing a variety of clinically important emm types.

Results and discussion

Erythromycin-resistant transconjugants were detected in 21/23 of the tested recipient strains, with mean frequencies of transconjugants ranging from $7.20 \times 10^{-8}$ to $1.31 \times 10^{-6}$ conjugant/recipient (Table 1). Statistical analysis showed that there was a significant difference in the conjugation frequency between strains (ANOVA, $P < 0.001$). PCR confirmed that all 85 randomly selected transconjugants were positive for the mef(A) gene as well as the 5′ and 3′ junctions of Tn1207.3-comEC. All ampliﬁcations were of the expected size. Thus, growth on erythromycin-selective plates can be taken as being strongly indicative of mef(A) acquisition by the previously susceptible, mef(A)-negative recipient strains and the integration of Tn1207.3 into the comEC locus.

The association of emm type with conjugative frequency is difficult to assess given the number of isolates of each emm type was low. However, the data show a trend for higher frequencies of conjugation among emm1 and emm4, seen in the region of $10^{-6}$ to $10^{-7}$ with lower

Table 1 Strain characteristics and gene transfer frequencies of the mef(A)-bearing Tn1207.3 element (SMH036) to macrolide-susceptible Streptococcus pyogenes

<table>
<thead>
<tr>
<th>emm type</th>
<th>Recipient</th>
<th>Multilocus sequence type</th>
<th>Site of isolation</th>
<th>Mean frequencies of three transfers per recipient ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>emm1</td>
<td>R366</td>
<td>ST28</td>
<td>Blood</td>
<td>$9.83 \times 10^{-6} \pm 1.87 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>R203</td>
<td>ST28</td>
<td>Blood</td>
<td>$1.13 \times 10^{-6} \pm 6.46 \times 10^{-7}$</td>
</tr>
<tr>
<td></td>
<td>R057</td>
<td>ST28</td>
<td>Eye</td>
<td>$3.86 \times 10^{-6} \pm 1.91 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>R502</td>
<td>Not available</td>
<td>Blood</td>
<td>$4.23 \times 10^{-6} \pm 1.85 \times 10^{-6}$</td>
</tr>
<tr>
<td>emm2</td>
<td>R837</td>
<td>ST55</td>
<td>Blood</td>
<td>$3.17 \times 10^{-6} \pm 3.52 \times 10^{-7}$</td>
</tr>
<tr>
<td></td>
<td>R007</td>
<td>ST55</td>
<td>Blood</td>
<td>$2.37 \times 10^{-6} \pm 5.30 \times 10^{-7}$</td>
</tr>
<tr>
<td>emm3</td>
<td>R032</td>
<td>ST315</td>
<td>Throat</td>
<td>$1.20 \times 10^{-7} \pm 1.52 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>R079</td>
<td>ST315</td>
<td>Throat</td>
<td>$9.80 \times 10^{-7} \pm 1.72 \times 10^{-7}$</td>
</tr>
<tr>
<td>emm4</td>
<td>R317</td>
<td>ST39</td>
<td>Blood</td>
<td>$4.50 \times 10^{-7} \pm 4.93 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>R211</td>
<td>ST39</td>
<td>Blood</td>
<td>$6.47 \times 10^{-7} \pm 1.05 \times 10^{-7}$</td>
</tr>
<tr>
<td></td>
<td>R101</td>
<td>ST39</td>
<td>Ear</td>
<td>$6.17 \times 10^{-6} \pm 3.75 \times 10^{-7}$</td>
</tr>
<tr>
<td>emm9</td>
<td>R480</td>
<td>ST75</td>
<td>Blood</td>
<td>$9.20 \times 10^{-7} \pm 1.89 \times 10^{-7}$</td>
</tr>
<tr>
<td>emm11</td>
<td>R097</td>
<td>ST75</td>
<td>Pus</td>
<td>$1.60 \times 10^{-7} \pm 1.53 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>R098</td>
<td>ST22</td>
<td>Blood</td>
<td>$8.13 \times 10^{-7} \pm 1.67 \times 10^{-1}$</td>
</tr>
<tr>
<td>emm12</td>
<td>R693</td>
<td>ST22</td>
<td>Blood</td>
<td>$3.67 \times 10^{-7} \pm 1.86 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>R214</td>
<td>ST36</td>
<td>Blood</td>
<td>$1.19 \times 10^{-7} \pm 5.01 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>R113</td>
<td>ST36</td>
<td>Throat</td>
<td>$3.97 \times 10^{-7} \pm 4.97 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>R044</td>
<td>ST36</td>
<td>Throat</td>
<td>$6.00 \times 10^{-7} \pm 8.88 \times 10^{-8}$</td>
</tr>
<tr>
<td>emm59</td>
<td>R205</td>
<td>Not available</td>
<td>Blood</td>
<td>$4.36 \times 10^{-6} \pm 8.60 \times 10^{-8}$</td>
</tr>
<tr>
<td>emm77</td>
<td>R054</td>
<td>ST77</td>
<td>Skin</td>
<td>$&lt;3.77 \times 10^{-8}$ (none detected)</td>
</tr>
<tr>
<td></td>
<td>R115</td>
<td>ST77</td>
<td>Vagina</td>
<td>$&lt;1.08 \times 10^{-8}$ (none detected)</td>
</tr>
<tr>
<td>emm81</td>
<td>R202</td>
<td>ST117</td>
<td>Blood</td>
<td>$3.97 \times 10^{-7} \pm 1.48 \times 10^{-7}$</td>
</tr>
<tr>
<td>emm87</td>
<td>R208</td>
<td>ST62</td>
<td>Blood</td>
<td>$7.20 \times 10^{-7} \pm 8.99 \times 10^{-8}$</td>
</tr>
</tbody>
</table>
rates seen particularly with the single isolates of emm59
and emm87, in the region of 10^{-9}, Table 1. These pat-
terns correlate well with the observed frequency of mef(A)
among clinical isolates belonging to these emm types, mef
(A) being highly prevalent among emm1 and emm4 iso-
lates but having not been reported among emm59 and
emm87 isolates (Creti et al. 2005; Grivea et al. 2006;
Silva-Costa et al. 2008; Wajima et al. 2013). These data
also highlight the potential for the emergence of mef (A)-mediated resistance in previously susceptible emm
types. Two isolates, both belonging to emm77, did not
produce transconjugants above the lower limit of detec-
tion, Table 1, suggesting that a low capacity for acquisi-
tion exists among emm77. This again correlates well with
epidemiological data, and emm77 has not previously been
associated with mef(A). Interestingly, previous work using
transduction also failed to transfer mefA into an emm 77
isolate (Di Luca et al. 2010). All seven emm types included
here and reported previously to harbour the mef
(A) gene were able to acquire it by conjugation in vitro.
Taking only the emm types for which at least three iso-
lates were included, emm1, emm4 and emm12, the higher
mean frequencies of transconjugants seen with emm1 and
emm4 isolates were statistically significant compared to
those seen with emm12 isolates. This suggests that an
association exists between emm type and the transfer fre-
cency of Tn1207.3 but the testing of a larger collection of
isolates is needed to explore this further.

Together the results demonstrate that significant vari-
ability exists in mef(A) gene conjugative transfer rates
between Strep. pyogenes strains and that this phenomenon
may contribute, at least in part, to the different frequen-
cies of mef(A) observed among clinical GAS strains. The
mechanisms responsible for these differing rates of mefA
transfer are not known, but M proteins have previously
been proposed to act as barriers for horizontal gene
exchange and thus may play a role (Schmitz et al. 2003).
Furthermore, we demonstrate potential for the develop-
ment of mef(A)-mediated erythromycin resistance in cur-
rently susceptible lineages.

Materials and methods

Bacterial isolates

Group A streptococci isolates were collected, as part of
a separate study, from two London Hospitals, St.
Mary’s and Paddington and Hammersmith, between
1993 and 2005 (McGregor and Spratt 2005). mef(A)
recipient strains belonged to the following eleven emm
types: emm1, emm2, emm3, emm4, emm9, emm11,
emm12, emm59, emm77, emm81 and emm89. Strains
were chosen based on their availability for study and
because they include some of the most common lin-
eages among clinical disease isolates in Europe (Steer
et al. 2009) (Table 1). They also include lineages with
observed differences in their frequency of mef(A) preva-
ience (Creti et al. 2005; Grivea et al. 2006; Silva-Costa
et al. 2008; Wajima et al. 2013). Isolate SMH036
(emm75) was used as the Tn1207.3 donor and carries
the Tn1207.3 element inserted into the comEC locus.
Discrimination of mef gene subclasses A, E, O and I by
RFLP analysis confirmed the presence of only mef(A) in
SMH036. SMH036 was confirmed to be resistant to
erthromycin with a MIC of 8 mg l^{-1} and grew readily
on 2 mg l^{-1} erythromycin-selective blood plates. It was,
however, susceptible to fusidic acid and rifampicin. All
recipient strains were confirmed to be susceptible to
erthromycin (MIC < 0.015–0.12 g l^{-1}) and unable to
grow on selective blood agar plates containing 2 mg l^{-1}
erthromycin used in screening for transconjugants. The
wild-type recipients were also confirmed to be suscepti-
ble to rifampicin and fusidic acid, and resistant deriva-
tives of these, used to differentiate transconjugants from
SMH036, were generated by inducing to spontaneous
mutations conferring resistance to rifampicin or fusidic
acid.

In vitro conjugative gene transfer of Tn1207.3

Filter mating was performed according to Giovanetti et al.
(Giovanetti et al. 2002). Transconjugants were selected on
blood agar plates containing either fusidic acid (25 mg l^{-1})
or rifampicin (25 mg l^{-1}) in addition to erythromycin
(2 mg l^{-1}). Transfers were performed in triplicate.

Confirmation of mef(A) transfer

Putative transconjugants were tested for the presence of
mef(A) by PCR using primers mefF/mefR as previously
described (Sutcliffe et al. 1996b). Both Tn1207.3/comEC
junctions were also examined by PCR, primers TnRJF/
TnRJR (D’Ercole et al. 2005) spanning the 5’ junction and
comECF/orfLF (Santagati et al. 2003) spanning the 3’
junction. Donor SMH036 was used as a positive control
for these PCRs. Transconjugants were randomly selected
(3–6 per donor/recipient combination) for PCR valida-
tion with a total of n = 85. All recipient strains produced
no amplicons in these PCRs.

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Conflict of interest

No conflict of interest declared.

References


