Predominance of dfrG as determinant of trimethoprim resistance in imported Staphylococcus aureus


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Abstract

To investigate the global occurrence of trimethoprim–sulfamethoxazole resistance and the genetic mechanisms of trimethoprim resistance, we analysed Staphylococcus aureus from travel-associated skin and soft-tissue infections treated at 13 travel clinics in Europe. Thirty-eight per cent (75/196) were trimethoprim-resistant and 21% (41/196) were resistant to trimethoprim–sulfamethoxazole. Among methicillin-resistant S. aureus, these proportions were 30% (7/23) and 17% (4/23), respectively. DfrG explained 92% (69/75) of all trimethoprim resistance in S. aureus. Travel to South Asia was associated with the highest risk of acquiring trimethoprim–sulfamethoxazole-resistant S. aureus. We conclude that globally dfrG is the predominant determinant of trimethoprim resistance in human S. aureus infection.

Keywords: Communicable diseases, emerging drug resistance, methicillin-resistant Staphylococcus aureus, molecular epidemiology, Panton–Valentine leukocidin, sentinel surveillance, staphylococcal skin infections, travel, trimethoprim–sulfamethoxazole combination

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The drastic stagnation in the development of novel antibacterial chemotherapies increasingly forces infectious diseases practitioners to resort to rediscovering ‘old antibiotics’ [1]. Trimethoprim–sulfamethoxazole has seen a renaissance for the treatment of skin and soft-tissue infections (SSTI) caused by methicillin-resistant S. aureus (MRSA) in Europe [2] and North America [3], where most isolates are susceptible. The recent observation that trimethoprim–sulfamethoxazole, in combination with rifampicin is non-inferior to linezolid for the treatment of severe staphylococcal infections [4] illustrates the renewed interest in, and potential of, antifolate compounds. However, research on imported S. aureus and from Africa strongly suggests that trimethoprim–sulfamethoxazole resistance is emerging around the globe [5–8], reaching up to 44% in South Asia [8].

Here, we analyse a geographically and genetically diverse collection of imported S. aureus from returnees treated for SSTI. We aim to describe the contribution of trimethoprim resistance and its genetic determinants towards the global upsurge in trimethoprim–sulfamethoxazole resistance in S. aureus causing human infections.

From May 2011 to December 2013, swab materials and patient information from travel-associated SSTI were collected at 13 travel clinics across Europe (www.staphtrav.eu) [8].

For comparison, we used consecutive S. aureus isolates from outpatients with acute onset SSTI treated at the Department of...
Dermatology in Heidelberg, between December 2013 and May 2014. Materials taken from patients with chronic wounds, hospitalization or travel outside Europe 3 months before the onset of SSTI were excluded.

Swab cultures. *S. aureus* species confirmation, antimicrobial susceptibility testing and testing for the meca and Panton–Valentin leukocidin (PVL) (lukS/lukF) genes by PCR were performed as described elsewhere [8]. Trimethoprim-resistant strains were tested for the presence of dfrA, dfrK and dfrG by PCR [6]. The intrinsic dfrB genes of trimethoprim-resistant strains without known dfr resistance genes were sequenced and analysed for possible chromosomal mutations [6]. The tetracycline resistance genes (tet(L), tet(K), tet(M) and tet(O)) were detected by PCR [9].

The study was approved by the Ethics Committee, Faculty of Medicine, Eberhard Karls Universität Tübingen and by the institutional review boards of the contributing centres, if necessary.

Of 318 submissions from independent SSTI cases [8], 62% (196/318) were caused by *S. aureus*. Thirty-eight per cent (75/196) of imported *S. aureus* were trimethoprim-resistant and 21% (41/196) were also resistant to a combination of trimethoprim–sulfamethoxazole. Twelve per cent (23/196) of all *S. aureus* isolates were MRSA. Of these, 17% (4/23) exhibited concomitant trimethoprim–sulfamethoxazole resistance and 30% (7/23) trimethoprim resistance, respectively.

Among 66 *S. aureus* strains collected from German patients with acute SSTI, there was one trimethoprim–sulfamethoxazole-resistant strain, but the remaining isolates were susceptible to trimethoprim and trimethoprim–sulfamethoxazole. Five per cent (3/66) of isolates in the control group were PVL+, and none was MRSA.

Imported *S. aureus* was more often resistant to trimethoprim and trimethoprim–sulfamethoxazole than isolates from autochthonous infections with the highest odds ratio estimates for imports from South Asia, followed by Africa, Latin America and South-East Asia (Table 1). Besides, trimethoprim and trimethoprim–sulfamethoxazole resistance was positively associated with abscess formation, presence of PVL-encoding genes, and particular spa types (Table 1).

Altogether dfrG accounted for 92% (69/75) of all trimethoprim resistance, whereas 7% (5/75) was due to dfrA, and only one strain carried the F98Y dfrB mutation. We did not find any dfrK-mediated trimethoprim resistance. Although dfrG was the predominant trimethoprim-resistance mechanism in isolates from Africa (38/40), South Asia (21/22) and South-East Asia (7/8) (Table 1), isolates from South America were found to also carry a substantial proportion of dfrA (2/5) next to dfrG (3/5) genes. Looking at all imported isolates, the presence of dfrG was not restricted to particular spa types, but, when compared with all other spa types, it was statistically more often present in t355, t021, t084 and t314 (Table 1).

DfrG-mediated trimethoprim resistance was associated with resistance to tetracycline and ciprofloxacin, but not with resistance to methicillin (Table 1). Of 23 isolates resistant to both tetracycline and trimethoprim with identifiable tetracycline-resistance genes, 20 carried a combination of dfrG and tet(K), two of dfrG and tet(M), and one isolate an F98Y mutated dfrB and tet(K). The common combination of dfrG and tet(K) (n = 20) clustered in spa t355 (n = 7) and t314 (n = 4), explaining all trimethoprim/tetracycline co-resistance observed within these genotypes.

One of 66 *S. aureus* in the control group was trimethoprim–sulfamethoxazole and penicillin-resistant, dfrG-positive, spa type t314; and isolated from an abscess (PVL+). Follow up with the treating physician did not reveal exposure abroad 3 months before onset of the SSTI.

Analysing isolates imported from six major regions of the world to Europe, we show that dfrG accounts for 92% of all trimethoprim resistance in *S. aureus* from human SSTI. Based on its global abundance, and by drawing on our previous and similar findings in *S. aureus* in Africa and in a limited number of isolates imported from there [6], we propose that dfrG is globally the most common genetic determinant of trimethoprim resistance in *S. aureus* causing human infection.

In the present study, the majority of trimethoprim-resistant *S. aureus* from South and South-East Asia harboured dfrG. This is in sharp contrast to one report of that region describing dfrG in MRSA causing a clonal outbreak in a hospital in Chiang Mai, Thailand [10]. Similarly, we found dfrG to be the predominant genetic determinant of trimethoprim resistance in isolates from Latin America. To the best of our knowledge, this is the first report of dfrG in *S. aureus* from human infection from that region. Therefore, and in conjunction with our published findings from Africa [6], we present ample evidence that dfrA and the F98Y mutation of the autochthonous dfrB gene, i.e. those genetic elements that are commonly referred to as the key genetic determinants of trimethoprim resistance [11,12] are, on a global scale, less commonly found than dfrG in trimethoprim-resistant *S. aureus* causing human infection.

In line with recent work that successfully demonstrated the location of dfrG on a mobile genetic element in *S. aureus* causing human infections [13], its presence in imports was not clonally restricted, further supporting its mobile nature. Interestingly, research in *Staphylococcus pseudintermedius* colonizing cats and dogs demonstrated that 90% of trimethoprim resistance in that species is dfrG-mediated [14], inviting a further hypothesis on a cross-species transfer, from animal to human staphylococci.

In our control group one *S. aureus* (spa type t314) was PVL+, trimethoprin–sulfamethoxazole resistant. On further scrutiny,
### TABLE I. Trimethoprim–sulfamethoxazole-resistance and trimethoprim-resistance in 196 *Staphylococcus aureus* isolates from imported skin and soft-tissue infections

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>n (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trimethoprim–sulfamethoxazole (TMP-SMZ)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Resistant</strong></td>
<td>41 (21)</td>
<td></td>
<td></td>
<td>75 (38)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Susceptible</strong></td>
<td>155 (79)</td>
<td></td>
<td></td>
<td>121 (62)</td>
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<tr>
<td><strong>Trimethoprim (TMP)</strong></td>
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</tr>
<tr>
<td><strong>Resistant</strong></td>
<td>40 (63)</td>
<td>108.3 (15.9–448.5)</td>
<td>&lt;0.0001</td>
<td>38 (40)</td>
<td>2.4 (0.9–5.9)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Susceptible</strong></td>
<td>24 (37)</td>
<td></td>
<td></td>
<td>2 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>dfr genes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>dfrG</strong></td>
<td>3 (4.0)</td>
<td></td>
<td></td>
<td>2.0 (0.9–4.2)</td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td><strong>dfrA</strong></td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>1 (20)</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td><strong>dfrB F98Y</strong></td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Co-resistance</strong></td>
<td></td>
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<tr>
<td><strong>Methicillin/mecA</strong></td>
<td>24 (17)</td>
<td>0.7 (0.3–1.3)</td>
<td>0.4</td>
<td>15 (11)</td>
<td>0.5 (0.2–1.2)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Any other non-β-lactam</strong></td>
<td>30 (23)</td>
<td>3.7 (2.0–6.7)</td>
<td>&lt;0.0001</td>
<td>20 (16)</td>
<td>3.1 (1.5–6.3)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Tetracycline</strong></td>
<td>40 (30)</td>
<td>3.1 (1.5–6.3)</td>
<td>0.002</td>
<td>22 (18)</td>
<td>3.1 (1.5–6.3)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>TetX</strong></td>
<td>32 (24)</td>
<td>3.9 (1.7–8.6)</td>
<td>0.001</td>
<td>16 (13)</td>
<td>3.9 (1.7–8.6)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Ciprofloxacin</strong></td>
<td>26 (19)</td>
<td>12.1 (4.0–37.0)</td>
<td>&lt;0.0001</td>
<td>21 (17)</td>
<td>12.1 (4.0–37.0)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are number (row %) of trimethoprim–sulfamethoxazole (TMP-SMZ) and trimethoprim (TMP) resistant isolates (left and central column) and number (row %) of TMP-resistant isolates carrying a respective dfr gene (right column). Odds ratios (OR), p-values, 95% confidence intervals (95% CI) from univariable logistic regression.

**Abbreviations:** PVL, Panton–Valentine leukocidin; meca, gene conferring methicillin resistance in *S. aureus*; dfrG/dfrA, accessory genes conferring high level trimethoprim resistance in *S. aureus*; dfrB, mutation F98Y of the autochthonous dehydrofolate-reductase-encoding gene conferring low-level trimethoprim resistance.

Only spa types with five or more isolates are displayed; ORs comparing the odds of TMP-SMZ or TMP resistance in *S. aureus* of a given type of lesion (i.e., ulcer, abscess, other type) with the odds of TMP-SMZ or TMP resistance among all remaining spa types combined.

Data for type of lesion missing for one patient; ORs comparing the odds of TMP-SMZ or TMP resistance in *S. aureus* lesions combined.

Only spa types (country of origin) of dfrG carrying MRSA where t148 (Coca Rica), t188 (Madagascar), t10437 (Coca Rica).

Only spa types (country of origin) of dfrA carrying MRSA where t314 (Coca Rica), t318 (Malaysia).

Only spa types (country of origin) of dfrB F98Y carrying MRSA where t148 (Coca Rica), t188 (Madagascar), t10437 (Coca Rica).

Only strains with tet(t) or tet(O) detected; n = 5 strains with undetected genetic determinant of tetracycline resistance.
there was no evidence of travel to tropical or subtropical regions in the last 3 months. *Staphylococcus aureus* spa t314 is one of the most common spa types found in African isolates [6,15], fostering speculations about trimethoprim–sulfamethoxazole-resistant clones being introduced and established in autochthonous populations.

We found co-resistances between anti-folate compounds and tetracycline. Tetracycline resistance is mediated by either tet(K) and tet(L) located on a plasmid or by tet(M) and tet(O) located on a transposon or integrated in the chromosome [16]. In any of these cases, transmission may be linked to other resistance genes, such as the dfr genes. Indeed, Kadlec and Schwarz have identified a plasmid-borne resistance gene cluster carrying dfr(K) and tet(L) in *S. aureus* ST398 from swine [17]. Furthermore, genome sequencing of a virulent MRSA strain ST239 revealed the co-localization of tet(M) and dfrG on a transposon Tn5801 [18]. Our observations on the strong association of dfrG with tet(K) in *S. aureus* causing human infection suggest that there may be a similar genetic link between these two resistance genes. This would pose a major threat to global public health, as both tetracycline and trimethoprim are resistance genes, such as the dfr genes. Indeed, Kadlec and Schwarz have identified a plasmid-borne resistance gene cluster carrying dfr(K) and tet(L) in *S. aureus* ST398 from swine [17].

Furthermore, genome sequencing of a virulent MRSA strain ST239 revealed the co-localization of tet(M) and dfrG on a transposon Tn5801 [18]. Our observations on the strong association of dfrG with tet(K) in *S. aureus* causing human infection suggest that there may be a similar genetic link between these two resistance genes. This would pose a major threat to global public health, as both tetracycline and trimethoprim–sulfamethoxazole are the two compounds of choice for the treatment of moderately severe SSTI caused by MRSA according to IDSA guidelines [3].

We observed a strong and statistically significant association between the presence of the genes encoding for PVL and trimethoprim–sulfamethoxazole resistance (Table 1). In extension to a recent report by Kraef et al. [19], we could reproduce this finding in immunocompetent patients and a clonally more diverse *S. aureus* population. Considering that dfrG in *S. aureus* is likely to be localized on a genetic element that may have potential to accept other resistance genes such as tet(K), its strong association with PVL is of concern because, under intermittent antibiotic pressure, the combined presence of virulence and resistance genes will be particularly efficient in propagating the hosting *S. aureus* clone.

In summary, we provide ample evidence that dfrG is globally the predominant genetic determinant of trimethoprim resistance in *S. aureus* causing human infection. Frequent import, location on a mobile genetic element, and a potential association with tet(K) are features of an emerging resistance gene that has the potential to rapidly limit our antimicrobial treatment options in the MRSA era.

**Transparency declaration**

All authors have stated that there are no conflicts of interest.

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**References**


