Emergence of trimethoprim resistance gene dfrG in Staphylococcus aureus causing human infection and colonization in sub-Saharan Africa and its import to Europe

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Objectives: Co-trimoxazole (trimethoprim/sulfamethoxazole) is clinically valuable in treating skin and soft tissue infections (SSTIs) caused by community-associated methicillin-resistant Staphylococcus aureus (MRSA). The genetic basis of emerging trimethoprim/sulfamethoxazole resistance in S. aureus from Africa is unknown. Such knowledge is essential to anticipate its further spread. We investigated the molecular epidemiology of trimethoprim resistance in S. aureus collected in and imported from Africa.

Methods: Five hundred and ninety-eight human S. aureus isolates collected at five locations across sub-Saharan Africa [Gabon, Namibia, Nigeria (two) and Tanzania] and 47 isolates from travellers treated at six clinics in Europe because of SSTIs on return from Africa were tested for susceptibility to trimethoprim, sulfamethoxazole and trimethoprim/sulfamethoxazole, screened for genes mediating trimethoprim resistance in staphylococci [dfrA (dfrS1), dfrB, dfrG and dfrK] and assigned to spa genotypes and clonal complexes.

Results: In 313 clinical and 285 colonizing S. aureus from Africa, 54% of isolates were resistant to trimethoprim, 21% to sulfamethoxazole and 19% to trimethoprim/sulfamethoxazole. We found that 94% of trimethoprim resistance was mediated by the dfrG gene. Of the 47 S. aureus isolates from travellers with SSTIs, 27 (57%) were trimethoprim resistant and carried dfrG. Markers of trimethoprim resistance other than dfrG were rare. The presence of dfrG genes in S. aureus was neither geographically nor clonally restricted.
Conclusions: dfrG, previously perceived to be an uncommon cause of trimethoprim resistance in human *S. aureus*, is widespread in Africa and abundant in imported *S. aureus* from ill returning travellers. These findings may foreshadow the loss of trimethoprim/sulfamethoxazole for the empirical treatment of SSTIs caused by community-associated MRSA.

Keywords: trimethoprim/sulfamethoxazole combination, sulphonamides, travel, gene transfer, horizontal, tetrahydrofolate dehydrogenase, drug resistance, microbial, methicillin-resistant *Staphylococcus aureus*, plasmids, skin diseases, infectious, soft tissue infections, public health surveillance, communicable diseases, emerging determinants of trimethoprim resistance contribute to the common trimethoprim/sulfamethoxazole resistance of *S. aureus* from humans on the African continent.

To this end, we conducted a multicentre cross-sectional study on trimethoprim resistance in existing *S. aureus* strain collections from locations in West, East, Southern and Central Africa and in staphylococci isolated at six travel clinics in Europe. Overall, we determined trimethoprim, sulfamethoxazole and trimethoprim/sulfamethoxazole resistance and screened for dfrB mutations and additional variant *dfr* genes (i.e. *dfrA*, *dfrG* and *dfrK*) in 645 *S. aureus* isolates. With this study, we aim to: (i) providing a comprehensive overview on the molecular epidemiology of trimethoprim resistance in *S. aureus* from sub-Saharan Africa; (ii) determining the relative contribution of different trimethoprim resistance genes in *S. aureus* towards altered susceptibility to trimethoprim/sulfamethoxazole; and (iii) assessing the threat of emerging trimethoprim/sulfamethoxazole resistance in *S. aureus* through the import of resistance genes into Europe.

Methods

Strain collections

**Gabon**

Colonizing (*n* = 100) and clinical isolates of *S. aureus* from various infections [skin and soft tissue (*n* = 100) and bloodstream (*n* = 12)] were collected between October 2010 and August 2012 from community dwellers in the region of Lambaréné (colonization samples) and from patients at the Albert Schweitzer Hospital, Lambaréné (clinical samples). Nasal swabs from asymptomatic subjects were taken applying the following exclusion criteria: (i) no hospitalization; or (ii) antibacterial or antituberculous treatment within 4 weeks prior to sampling. Clinical samples were obtained from patients with community-onset disease defined as a positive *S. aureus* culture from samples taken ≤48 h of admission.

**Namibia**

Between November 2008 and December 2009, 15 colonizing and 101 clinical *S. aureus* isolates from various infections [skin and soft tissue (*n* = 31), urinary tract (*n* = 19), respiratory tract (*n* = 37), ear (*n* = 7), eye (*n* = 4) and bloodstream (*n* = 3)] were obtained from diagnostic material of patients hospitalized either in the University Clinic of Windhoek or the Oshakati Teaching Hospital.

**Nigeria I**

Between January and April 2009, 59 clinical isolates from various infections [skin and soft tissue (*n* = 32), urinary tract (*n* = 9), ear (*n* = 7),
unknown site (n = 4), oropharynx (n = 3), eye (n = 3) and bloodstream (n = 1) were obtained from samples processed in the microbiology laboratories of referral healthcare institutions in Ile-Ife, Ibadan and Lagos in south-west Nigeria and Maiduguri in north-east Nigeria.13

Nigeria II

Between December 2008 and August 2010, single nasal swabs were collected from the anterior nares of 374 adult outpatients from two HIV clinics in Lagos, Nigeria. During the same period, 370 apparently healthy adult individuals working or studying at institutions of higher education were enrolled. From this collection, a total of 95 colonizing S. aureus from HIV-positive and 29 from HIV-negative individuals were randomly selected for this study.

Tanzania

In Bagamoyo district, from January 2011 to October 2012, colonizing (n = 46) and clinical isolates of S. aureus from various infections (skin and soft tissue (n = 39) and bloodstream (n = 2)) were collected from asymptomatic community dwellers and patients at the district hospital and four healthcentres (Kiwangwa, Yombo, Mapinga and Mokurunge). Samples were taken applying the same inclusion and exclusion criteria as in Gabon.29

Returning travellers

Since June 2011, the European Network on Imported Staphylococcus aureus (StaphTrav) systematically collects S. aureus from ill returning travellers (www.staphtrav.eu). In brief, participating travel clinics submit lesional swabs from returnees suffering from SSTIs during or 30 days after a journey outside Europe. By August 2013, the database contained a total of 250 anonymized submissions. Of these, 47 were S. aureus from independent cases with SSTIs on return from sub-Saharan Africa. Contributing centres were as follows: Tübingen, Paul-Lechler-Krankenhaus (n = 12); Tübingen, Institute of Tropical Medicine (n = 11); Berlin (n = 9); Rotterdam (n = 6); Amsterdam (n = 5); and Paris (n = 4).

Pre-travel subjects

From May 2010 to March 2011, 81 S. aureus isolates from single nasal swabs were obtained from 300 individuals consulting the University of Tübingen travel clinic for pre-travel advice. Exclusion criteria were, within 12 months prior to sampling, hospitalization, healthcare-related occupation or travel outside Europe. This sample was chosen to represent the population of pre-travel colonizing staphylococci as a cause of SSTIs while travelling and to test the hypothesis of S. aureus acquisition abroad. Given that staphylococci causing lesions in travellers were not acquired abroad, one would expect to find similar resistance phenotypes and gene content in S. aureus from pre-travel and post-travel consultations.

Ethics statement

This research was conducted at the Institute of Tropical Medicine in Tübingen and used S. aureus from existing strain collections together with anonymized data on type of infection or colonization. Initial sample and data collection from African and European volunteers and ill returning travellers included written informed consent and had been approved by the competent ethics committees in Africa and Europe. S. aureus causing infection in Africa were collected from routine diagnostic material, either after approval by the competent ethics committees in Gabon, Tanzania and Nigeria (Collection II) or as part of surveillance activities at hospitals in Nigeria (Collection I) and Namibia.

Antimicrobial susceptibility testing

The agar disc diffusion susceptibility testing (Kirby–Bauer) was recommended as the CLSI and EUCAST using S. aureus ATCC 25923 as a control. In brief, overnight cultures were adjusted to 0.5 McFarland and cultured on EUCAST and CLSI-conforming Mueller–Hinton agar (Oxoid, Wesel, Germany). The zone of inhibition was measured after 18–24 h of incubation at 37 °C. All isolates were selected for resistance to trimethoprim (5 µg), sulfamethoxazole (23.75 µg), and trimethoprim/sulfamethoxazole combined (1.25 µg/23.75 µg) (BD Diagnostics, Heidelberg, Germany). The zones of inhibition for trimethoprim and trimethoprim/sulfamethoxazole were interpreted according to EUCAST clinical breakpoints. As there are no EUCAST clinical breakpoints for sulfamethoxazole as a monagent for S. aureus, those of the CLSI were used instead.

DNA isolation

Three to four S. aureus colonies were picked from overnight cultures grown on Columbia agar with 5% sheep’s blood, suspended in TE buffer pH 8.0 and incubated with 30 µg/mL lysostaphin at 37 °C for 30 min prior to DNA isolation with the Qiagen Blood and Tissue Kit (Qiagen GmbH, Germany) according to the manufacturer’s protocol.

Genetic characterization

All isolates were characterized by multiplex PCR for the presence of mecA (forward GTAGAAATGACTGAACGTCCGATAA, reverse CCAATTCCACATTGTTCCGCTTAA) and coa (forward CGAGACCAAGATTCAACAAG, reverse AAAGAAAACCACTCACATCA) genes. All strains were genotyped based on partial sequencing of the S. aureus protein A (spa) and assigned to spa types using the Staph Type 2.1.1 software (Ridam, Münster, Germany). spa Types were clustered into spa clonal complexes (CCs) using the Based Upon Repeat Pattern algorithm as implemented in StaphType software with parameters set to exclude spa types if repeats <5 and to cluster these if cost ≤4.

dfr screening and dfrB sequencing

All isolates were screened for known dfr genes in S. aureus by conventional PCR with an annealing temperature of 57 °C using published primers. For all trimethoprim-resistant S. aureus without detectable dfrA, dfrG or dfrK genes, the DHFR-encoding dfrB was amplified using the primer set dfrB1 (5′-AAATTGTGTTAAATTAAAGATAACTT-3′) and dfrB2 (5′-TAAGTATTCTTTAGAAAACTCCATTACCCATCA). The f98Y mutation. In addition, we compared the dfrB sequences of a random sample of 172 trimethoprim-susceptible and 78 trimethoprim-resistant S. aureus to explore for potential associations between other sequence variants and the resistance phenotype.

Results

Overall, 598 human S. aureus isolates from four countries in sub-Saharan Africa were analysed (Table 1). Of these, 324 (54.2%) were resistant to trimethoprim, 127 (21.2%) to sulfamethoxazole and 114 (19.1%) to a combination of trimethoprim and sulfamethoxazole. Resistance to trimethoprim/sulfamethoxazole was only found in strains that were fully resistant to both trimethoprim and sulfamethoxazole. Accordingly, intermediate resistance to sulfamethoxazole in the presence of full trimethoprim resistance was not sufficient to render S. aureus resistant to co-trimoxazole. Similarly, all S. aureus isolates that were resistant to either trimethoprim or sulfamethoxazole whilst being susceptible or showing
Table 1. Trimethoprim, sulfamethoxazole and trimethoprim/sulfamethoxazole resistance in S. aureus from Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Type</th>
<th>Total no. of strains</th>
<th>TMP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total no. of spa types&lt;sup&gt;c&lt;/sup&gt;</th>
<th>meca&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SMZ&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SXT&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>Gabon</td>
<td>colonizing, n = 100</td>
<td>100</td>
<td>4 (4%)</td>
<td>6 (6%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td></td>
<td>SSTIs, n = 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>other, n = 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>resistant, n = 104</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>susceptible, n = 108</td>
<td></td>
<td>14 (13%)</td>
<td>7 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>Namibia</td>
<td>colonizing, n = 15</td>
<td>15</td>
<td>1 (3%)</td>
<td>20 (59%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td></td>
<td>SSTIs, n = 31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>other, n = 70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>resistant, n = 34</td>
<td></td>
<td>3 (4%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>susceptible, n = 82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>colonizing, n = 124</td>
<td>124</td>
<td>3 (2%)</td>
<td>85 (55%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td></td>
<td>SSTIs, n = 32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>other, n = 27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>resistant, n = 154</td>
<td></td>
<td>3 (2%)</td>
<td>85 (55%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td></td>
<td>susceptible, n = 29</td>
<td></td>
<td>3 (10%)</td>
<td>2 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>Tanzania</td>
<td>colonizing, n = 46</td>
<td>46</td>
<td>2 (6%)</td>
<td>5 (16%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SSTIs, n = 39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>other, n = 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>resistant, n = 32</td>
<td></td>
<td>5 (9%)</td>
<td>2 (4%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>susceptible, n = 55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>colonizing, n = 285</td>
<td>285</td>
<td>10 (3%)</td>
<td>116 (36%)</td>
<td>5 (2%)</td>
</tr>
<tr>
<td></td>
<td>SSTIs, n = 202</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>other, n = 111</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>resistant, n = 324</td>
<td></td>
<td>10 (3%)</td>
<td>116 (36%)</td>
<td>5 (2%)</td>
</tr>
<tr>
<td></td>
<td>susceptible, n = 274</td>
<td></td>
<td>10 (3%)</td>
<td>116 (36%)</td>
<td>5 (2%)</td>
</tr>
</tbody>
</table>

**Concomitant antifolate resistance**

**Trimethoprim resistance gene**

**Most frequent spa<sup>c</sup> types per stratum; numbers in brackets are numbers of strains with the given spa type.

**Total no. of spa types**

**meca**

**Notes:**

<sup>a</sup>Using EUCAST clinical breakpoints.

<sup>b</sup>Using CLSI clinical breakpoints.

<sup>c</sup>spa types with the five most common frequencies per stratum; numbers in brackets are numbers of strains with the given spa type.

<sup>d</sup>Excluding one non-typeable isolate.

<sup>e</sup>n = 95 from HIV-positive individuals.

**Abbreviations:**

TMP, trimethoprim; SMZ, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole; I, intermediate resistance; R, resistant; other, clinical infections other than SSTIs.
Trimethoprim resistance in *S. aureus* from Africa

Intermediate resistance to the other were susceptible to trimethoprim/sulfamethoxazole.

At least one molecular marker of resistance was identified in all trimethoprim-resistant isolates [Table 1 and Figure S1 (available as Supplementary data at JAC Online)]. Among these, the dfrG gene largely predominated (94\%) and, overall, occurred in half (304/598) of the *S. aureus* isolates from sub-Saharan Africa. In Gabon, Nigeria and Tanzania, 96\%–100\% of trimethoprim-resistant *S. aureus* harboured dfrG. Notably, in trimethoprim-resistant *S. aureus* from Namibia, dfrG prevailed (20/34), but dfrA was also common (14/34). One trimethoprim-resistant strain from Gabon (spa t1476, spa CC064) was positive for dfrG and dfrK genes concomitantly and two trimethoprim-resistant isolates (spa t1045, singleton) from Gabon were dfrA, dfrG and dfrK negative, but positive for the F98Y mutation in dfrB. None of the 274 trimethoprim-susceptible strains isolated from Africans harboured variant dfr genes. Of these, 172 (63\%) were also sequenced for dfrB and none carried the F98Y mutation. Several mutations in dfrB other than F98Y could be identified in trimethoprim-resistant and -susceptible *S. aureus* isolates alike (data not shown).

There was no evidence of dfrG being restricted to particular *S. aureus* clones. *S. aureus* of all major spa CCs (except for spa CC939 and spa CC1510) harboured dfrG (Figure 1). Likewise, individual spa types and the presence of dfrG did not correlate (Figure S2, available as Supplementary data at JAC Online).

Overall, 32 isolates of MRSA were identified (Table 2). Of these, 75\% were also resistant to trimethoprim/sulfamethoxazole. Similar to the findings in methicillin-susceptible *S. aureus* (MSSA), dfrG was present in trimethoprim-resistant MRSA from heterogeneous genetic backgrounds. The presence of dfrA in *S. aureus*, in contrast, clustered in MRSA spa CC064 (t064, t104 and t1443) from Namibia (n = 10) and Nigeria (n = 1), thus accounting for 11 out of 17 (65\%) dfrA detected in this study.

More than half of *S. aureus* isolated from travellers returning from sub-Saharan Africa and suffering from SSTIs were trimethoprim resistant and also carried dfrG (Table 3). In contrast, none of 81 *S. aureus* isolates collected from nares during pre-travel consultations was trimethoprim resistant. Trimethoprim resistance caused by dfrG was more likely to be present in *S. aureus* from returnees compared with isolates from pre-travel consultations (χ² test, P < 0.0001).

### Table 2. Trimethoprim, sulfamethoxazole and trimethoprim/sulfamethoxazole resistance in African MRSA

<table>
<thead>
<tr>
<th>Country</th>
<th>Swab type</th>
<th>MRSA with concomitant resistance toa</th>
<th>spa typesb,c</th>
<th>TMP resistance geneb,d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabon</td>
<td>colonizing</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSTIs, n=1</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Namibia</td>
<td>SSTIs, n=3</td>
<td>2 (67%)</td>
<td>t064 (2), t104 (1)</td>
<td>dfrA (1)</td>
</tr>
<tr>
<td>other, n=8</td>
<td></td>
<td>8 (100%)</td>
<td>t104 (4), t064 (2), t1443 (2)</td>
<td>dfrA (8)</td>
</tr>
<tr>
<td>Nigeria</td>
<td>colonizing, n=6</td>
<td>6 (100%)</td>
<td>t064 (2), t008 (1), t037 (1), t197 (1), t7798 (1)</td>
<td>dfrA (6)</td>
</tr>
<tr>
<td>SSTIs, n=6</td>
<td></td>
<td>5 (83%)</td>
<td>t037 (3), t002 (1), t064 (1), t451 (1)</td>
<td>dfrA (6)</td>
</tr>
<tr>
<td>other, n=4</td>
<td></td>
<td>3 (75%)</td>
<td>t037 (2), t008 (1), t064 (1)</td>
<td>dfrA (3), dfrA (1)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>no MRSA strains</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>n=32</td>
<td>24 (75%)</td>
<td>t063 (1)</td>
<td>dfrA (12), dfrG (16)</td>
</tr>
</tbody>
</table>

TMP, trimethoprim; SMZ, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole; NA, not applicable; other, clinical infections other than SSTIs.

*aStrains with intermediate-level resistance were classified as susceptible.

*bUsing EUCAST clinical breakpoints.

*cUsing CLSI clinical breakpoints.

*dBold formatting indicates that the spa type contains dfrA and underlining indicates that the spa type contains dfrG.

*eNumbers in brackets are numbers of strains with the given spa type or harbouring the respective gene.

![Figure 1. Distribution of dfrG in *S. aureus* of the 10 most common spa CCs.](image-url)
**Table 3.** Trimethoprim, sulfamethoxazole and trimethoprim/sulfamethoxazole resistance in *S. aureus* from SSTIs in travellers returning from sub-Saharan Africa and treated in six travel clinics in Germany, France and the Netherlands

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of strains</th>
<th>Phenotypic antifolate resistance&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TMP resistance gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Southern Africa&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>West Africa&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15</td>
<td>11 (73%)</td>
<td>0</td>
</tr>
<tr>
<td>East Africa&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21</td>
<td>10 (48%)</td>
<td>0</td>
</tr>
<tr>
<td>Central Africa&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7</td>
<td>5 (71%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>27 (57%)</td>
<td>0</td>
</tr>
<tr>
<td>Pre-travel subjects&lt;sup&gt;f&lt;/sup&gt;</td>
<td>81</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>EUCAST clinical breakpoints were used for the trimethoprim and trimethoprim/sulfamethoxazole resistance phenotypes; CLSI clinical breakpoints were used to define sulfamethoxazole resistance.

<sup>b</sup>Zambia (n=3) and South Africa (n=1).

<sup>c</sup>Cape Verde (n=1), Senegal (n=2), Gambia (n=2), Guineo-Bissau (n=1), Sierra Leone (n=2), Ghana (n=4), Togo (n=1) and Nigeria (n=2).

<sup>d</sup>Kenya (n=8), Sudan (n=1), Rwanda (n=1), Uganda (n=4), Tanzania (n=3), Mozambique (n=2) and Malawi (n=2).

<sup>e</sup>Cameroon (n=5) and Democratic Republic of Congo (n=2).

<sup>f</sup>*S. aureus* collected from the nares of 300 subjects during pre-travel consultations.

<sup>g</sup>These nine isolates with altered susceptibility to trimethoprim belong to eight different spa types.

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**Discussion**

We show that *dfrG* is abundant and the most common genetic source of trimethoprim resistance in MSSA and MRSA from sub-Saharan Africa. We also demonstrate that *dfrG* can be frequently found in *S. aureus* from travellers returning with SSTIs from the African continent, but not in *S. aureus* colonizing travellers before departure. So far, *dfrG* has been perceived to play a minor role in human *S. aureus* infections based on its isolated occurrence in nasocomial outbreaks of MRSA in London and Chiang Mai, Thailand, and its otherwise sporadic description in staphylococci from animals only. Moreover, our results strongly suggest importation of *dfrG* genes through long-distance travel.

The *dfrG* gene was detected in MSSA and MRSA from a wide range of genotypes and CCs (Figure 1 and Figure S2), suggesting that it is located on a mobile genetic element. Indeed, successful transfer of plasmid-encoded *dfrG* was recently demonstrated in *Listeria monocytogenes*. Based on similar findings for *dfrA* and *dfrK*, we speculate that *dfrG* in *S. aureus* may also be located on a mobile element, thus allowing its swift horizontal transfer between different *S. aureus* lineages. The characteristics of this element, however, remain to be determined in detail.

We show that *dfrG* can be found in *S. aureus* belonging to those spa CCs that also harbour globally important community-associated MRSA lineages such as multilocus sequence type CC5, CC8 and CC30. Moreover, our observation on the importation of *dfrG* in *S. aureus* from ill returning travellers and previous descriptions of *dfrG* in MRSA causing hospital outbreaks underline the potential for future spread of *dfrG* within MRSA in populations where antifolate resistance is currently considered to be low, such as Europe and North America.

The *dfrA* gene accounted for 5% of trimethoprim resistance in our collection and clustered in MRSA spa CC064 from Namibia, suggesting inclusion of a locally circulating clone and thus underestimation of *dfrA* prevalence in *S. aureus* from sub-Saharan Africa in this study. Genetic sources of trimethoprim resistance other than *dfrG* and *dfrA* were rarely found among the *S. aureus* isolates from sub-Saharan Africa. The *dfrB* F98Y mutation previously described to confer intermediate-level trimethoprim resistance in *S. aureus* from Europe and Brazil accounted for <1% of trimethoprim resistance in sub-Saharan Africa.

For the first time, we detected *dfrK* in *S. aureus* outside of Europe, in one MSSA isolate (spa t1476, spa CC064, multilocus sequence type ST8) causing SSTIs in a subject from Gabon. To date, *dfrK* has been mainly found in livestock-associated *S. aureus* multilocus sequence type ST398 from Europe, occasionally together with *dfrG*, as reported here. Our findings suggest that *dfrK* can be integrated into *S. aureus* of other genetic backgrounds as well and may thus be of more general importance in determining trimethoprim resistance in staphylococci. Of note, trimethoprim/sulfamethoxazole-resistant MSSA spa t1476 has previously been reported from pigs in Senegal, putting our findings in line with existing evidence on the transmission of *S. aureus* harbouring trimethoprim resistance genes between humans and farm animals.

In this study, genetic screening for the additional *dfr* genes A, G and K or mutated *dfrB* fully explained the observed variation in trimethoprim susceptibility in *S. aureus* from sub-Saharan Africa. Moreover, we noted that sequencing did not reveal any *dfrB* mutation consistently associated with trimethoprim resistance other than known F98Y, a finding in line with a previous report. Overall, these results suggest that genetic screening for the...
presence of dfrG, dfrK, dfrA and the F98Y mutation in dfrB reliably identifies trimethoprim resistance in human \textit{S. aureus}, a conclusion that, once replicated in other populations, may be of value for the development of new molecular diagnostic tools.

The abundance of dfrG in \textit{S. aureus} from human infection has major implications for the development of new antifolate antibiotics. In vitro drug testing of candidate compounds is often performed against \textit{S. aureus} carrying dfrB mutations or dfrA only, an approach based on the prevailing view of their key role in determining trimethoprim resistance. Such testing, however, may not warrant activity against \textit{S. aureus} carrying other dfr genes. Indeed, one study reported that \textit{S. aureus} carrying dfrG was resistant against derivatives of a new family of antifolate compounds while strains carrying dfrA or dfrB (F98Y) were killed at low concentrations. Together with knowledge on substantial sequence variation between dfrG and dfrA, these findings suggest that the resistance mechanism of dfrG- and dfrK-encoded DHFR differs from that of S1-DHFR (dfrA) and SaDHFR (F98Y). In light of the abundance of dfrG in \textit{S. aureus} causing human infection reported here, in vitro antimicrobial testing of candidate antifolate compounds is recommended to include \textit{S. aureus} expressing dfrG- or dfrK-encoded DHFR.

Our results illustrate that both trimethoprim and sulfamethoxazole resistance are necessary for the manifestation of trimethoprim/sulfamethoxazole resistance. Hence, in regions with widespread presence of dfrG in \textit{S. aureus}, further emergence of co-trimoxazole resistance will be driven by changes in the prevalence of sulfamethoxazole resistance and vice versa. In this context, the fact that 11% of isolates from German control subjects showed altered sulfamethoxazole resistance (Table 3) is alarming, since the introduction of dfrG into this or similar populations may lead to a swift increase in phenotypic trimethoprim/sulfamethoxazole resistance. Therefore, research to elucidate the prevalence and mechanisms of sulfamethoxazole resistance in \textit{S. aureus} on a global scale is needed. Besides, these findings illustrate that the current practice of screening for resistance against trimethoprim/sulfamethoxazole, rather than screening for resistance to trimethoprim and sulfamethoxazole separately, greatly hampers surveillance, thus allowing antimicrobial resistance to emerge silently.

We would like to emphasize that, due to the heterogeneity of populations studied and a variable degree of clonality within the samples analysed, this study is not suitable to compare the between-country prevalence of \textit{S. aureus} antifolate resistance in sub-Saharan Africa. For example, half of the sample studied from Nigeria consisted of colonizing \textit{S. aureus} from HIV-infected individuals, explaining the comparably higher prevalence of sulfamethoxazole resistance in \textit{S. aureus} on a global scale is needed. Besides, these findings illustrate that the current practice of screening for resistance against trimethoprim/sulfamethoxazole, rather than screening for resistance to trimethoprim and sulfamethoxazole separately, greatly hampers surveillance, thus allowing antimicrobial resistance to emerge silently.

In summary, there is widespread presence of dfrG in \textit{S. aureus} from sub-Saharan Africa. Together with this first report on its abundance in \textit{S. aureus} from ill returning travellers, our observations allow suggesting further spread of trimethoprim resistance on a global scale and are alarming since they may foreshadow the upcoming loss of trimethoprim/sulfamethoxazole as a therapeutic option for the empirical treatment of SSTIs in the community-associated MRSA era. Further research and surveillance activities are urgently needed to safeguard the future effectiveness of co-trimoxazole in treating human infections and to design new antifolate antimicrobials with activity against dfrG-encoded DHFR. Considering that dfrG can be found in staphylococci from animal origin, speculations on the exchange of resistance genes between human- and livestock-associated \textit{S. aureus} seem plausible and raise serious concerns about the common use of trimethoprim in livestock husbandry.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 and Figure S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


