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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

Introduction

Morbidity due to *Staphylococcus aureus* infections is increasing around the globe[1,2] and the treatment of affected patients is complicated by the emergence of antimicrobial resistance.[3] Co-trimoxazole is a fixed-dose combination of the antifolate compounds trimethoprim and sulfamethoxazole, which act synergistically by inhibiting distinct steps in the bacterial folic acid synthesis. Currently, co-trimoxazole is valuable for the empirical antibiotic treatment of skin and soft tissue infections (SSTIs) caused by community-associated methicillin-resistant *S. aureus* (MRSA).[4–7]

In contrast to Europe where trimethoprim/sulfamethoxazole resistance in *S. aureus* is very rare,[8] up to 55% of colonizing and 72% of clinical *S. aureus* isolates from Africa are resistant to co-trimoxazole.[9–13] In general, this difference is attributed to a more frequent use of antifolate compounds for the treatment and prevention of infections in sub-Saharan Africa.[9,14] Recently, we observed that trimethoprim/sulfamethoxazole resistance is common in *S. aureus* causing SSTIs in travellers returning from the African continent.[15]

Two genetic mechanisms of trimethoprim resistance in *S. aureus* are known: (i) mutation of the chromosomal dihydrofolate reductase (DHFR) gene (*dfrB*); and (ii) resistance genes that encode variant DHFRs, some of which are known to be located on exchangeable genetic elements.[16–19] With regard to mutation of the autochthonous *dfrB* gene in *S. aureus*, a single functional mutation at position 98 (F98Y) confers intermediate-level trimethoprim resistance (MIC ≤256 mg/L).[16] In contrast, acquired *dfr* gene variants mediate high-level resistance to trimethoprim (MIC ≥512 mg/L). To date, three such genes have been identified in *S. aureus* of human origin: *dfrA* (*dfrS1*), *dfrG* and *dfrK*.[17–19]

In *S. aureus* isolated from humans, the *dfrB* F98Y mutation and *dfrA* are currently considered to be key determinants of trimethoprim resistance.[20–23] In contrast, *dfrG* is perceived as being rare in *S. aureus* from humans,[21] but to play a role in determining trimethoprim resistance in particular clones of *Staphylococcus pseudintermedius*[24] and *S. aureus* of animal origin.[25] In 2005, Sekiguchi *et al*.[19] for the first time reported on *dfrG* in an MRSA clone in a hospital in Chiang Mai, Thailand. More recently, an outbreak of MRSA exhibiting *dfrG* occurred in a London hospital.[26] The third DHFR variant, *dfrK*, has been identified predominantly in livestock-associated MRSA from Europe and only sporadically in humans.[27,28]

Comprehensive studies on the genetic basis of trimethoprim resistance in human *S. aureus* from Africa are missing. Interestingly, one study from Nigeria failed to detect the supposedly common *dfrA* gene,[13] suggesting that other genetic determinants of trimethoprim resistance contribute to the common trimethoprim/sulfamethoxazole resistance of *S. aureus* from humans on the African continent.[9,13]

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.[29]

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.[9] 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 Makurunge). 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 performed as recommended by 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 tested 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 monoagent 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 CCAATTCCACATTG TTTCGGTCTAA) 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 (Ridom, Münster, Germany).[30] *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.[31]

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.[25] 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′-AATTGTGTTAAATTAAAGATAACTT-3′) and dfrB2 (5′-TAAGTATTCTTTAGA TAAATCGGAT-3′), sequenced and then aligned with ATCC 25923 to identify 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

			Concomitant antifolate resistance										
			SMZ[b]		SXT[a]		Trimethoprim resistance gene						
Country	Type	TMP[a]	I	R	I	R	*dfrA*	*dfrG*	*dfrK+G*	*dfrB* (F98Y)	Most frequent *spa* types[c]	Total no. of *spa* types	*mecA*
Gabon	colonizing, *n*=100 SSTIs, *n*=100 other, *n*=12	resistant, *n*=104	4 (4%)	6 (6%)	2 (2%)	6 (6%)	1 (1%)	100 (96%)	1 (1%)	2 (2%)	t084 (40), t355 (34), t311 (4), t1476 (3), t1045 (2), t190 (2), t279 (2), t314 (2)	23[d]	1 (1%)
		susceptible, *n*=108	14 (13%)	7 (6%)	0	0	0	0	0	0	t939 (19), t355 (16), t1931 (6), t127 (4), t314 (4)	48	4 (4%)
Namibia	colonizing, *n*=15 SSTIs, *n*=31 other, *n*=70	resistant, *n*=34	1 (3%)	20 (59%)	1 (3%)	20 (59%)	14 (41%)	20 (59%)	0	0	t064 (8), t1476 (6), t104 (5), t062 (4), t1443 (2), t1774 (2), t891 (2)	12[d]	11 (32%)
		susceptible, *n*=82	3 (4%)	0	0	0	0	0	0	0	t318 (22), t084 (10), t267 (5), t148 (4), t021 (3), t085 (3), t12614 (3), t375 (3)	30	0
Nigeria	colonizing,[e] *n*=124 SSTIs, *n*=32 other, *n*=27	resistant, *n*=154	3 (2%)	85 (55%)	2 (1%)	83 (54%)	2 (1%)	152 (99%)	0	0	t084 (26), t064 (21), t3772 (17), t037 (6), t311 (5), t318 (5)	49	16 (11%)
		susceptible, *n*=29	3 (10%)	2 (7%)	0	0	0	0	0	0	t355 (5), t1045 (3), t084 (2), t127 (2), t311 (2), t939 (2)	19	0
Tanzania	colonizing, *n*=46 SSTIs, *n*=39 other, *n*=2	resistant, *n*=32	2 (6%)	5 (16%)	0	5 (16%)	0	32 (100%)	0	0	t002 (6), t1476 (4), t272 (3), t084 (2), t148 (2), t701 (2)	19	0
		susceptible, *n*=55	5 (9%)	2 (4%)	0	0	0	0	0	0	t1849 (4), t314 (3), t4198 (3), t084 (2), t127 (2), t2949 (2), t304 (2), t318 (2), t4499 (2), t941 (2)	41	0
Total	colonizing, *n*=285 SSTIs, *n*=202 other, *n*=111	resistant, *n*=324	10 (3%)	116 (36%)	5 (2%)	114 (35%)	17 (5%)	304 (94%)	1 (<1%)	2 (1%)	t084 (69), t355 (36), t064 (27), t3772 (18), t1476 (14)	80	28 (9%)
		susceptible, *n*=274	25 (9%)	11 (4%)	0	0	0	0	0	0	t318 (28), t939 (22), t355 (21), t084 (13), t314 (9)	114	4 (1%)

TMP, trimethoprim; SMZ, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole; I, intermediate resistance; R, resistant; other, clinical infections other than SSTIs.
[a]Using EUCAST clinical breakpoints.
[b]Using CLSI clinical breakpoints.
[c]*spa* types with the five most common frequencies per stratum; numbers in brackets are numbers of strains with the given *spa* type.
[d]Excluding one non-typeable isolate.
[e]*n*=95 from HIV-positive individuals.

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).

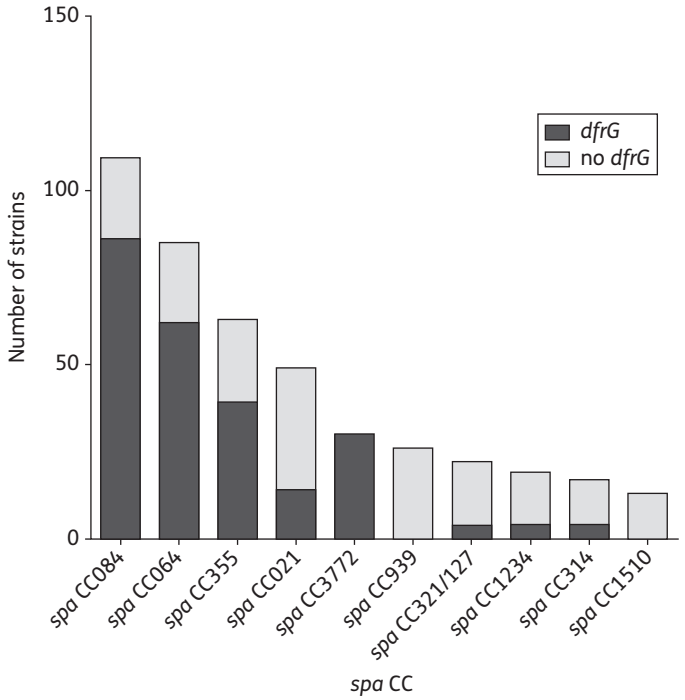

Figure 1. Distribution of *dfrG* in *S. aureus* of the 10 most common *spa* CCs.

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 (χ^2 test, $P<0.0001$).

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

Country	Swab type	MRSA with concomitant resistance to[a]			*spa* types[d,e]	TMP resistance gene[e]
		SXT[b]	TMP[b]	SMZ[c]		
Gabon	colonizing, $n=1$	0	0	0	t653 (1)	NA
	SSTIs, $n=4$	0	1 (25%)	0	t121 (2), **t186** (1), t653 (1)	*dfrA* (1)
Namibia	SSTIs, $n=3$	2 (67%)	3 (100%)	2 (67%)	**t064** (2), **t104** (1)	*dfrA* (2), *dfrG* (1)
	other, $n=8$	8 (100%)	8 (100%)	8 (100%)	**t104** (4), **t064** (2), **t1443** (2)	*dfrA* (8)
Nigeria	colonizing, $n=6$	6 (100%)	6 (100%)	6 (100%)	t064 (2), t008 (1), t037 (1), t197 (1), t7798 (1)	*dfrG* (6)
	SSTIs, $n=6$	5 (83%)	6 (100%)	5 (83%)	t037 (3), t002 (1) t064 (1), t451 (1)	*dfrG* (6)
	other, $n=4$	3 (75%)	4 (100%)	3 (75%)	t037 (2), **t008** (1), t064 (1)	*dfrG* (3), *dfrA* (1)
Tanzania	no MRSA strains					
Total	$n=32$	24 (75%)	28 (88%)	24 (75%)		*dfrA* (12) *dfrG* (16)

TMP, trimethoprim; SMZ, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole; NA, not applicable; other, clinical infections other than SSTIs.
[a]Strains with intermediate-level resistance were classified as susceptible.
[b]Using EUCAST clinical breakpoints.
[c]Using CLSI clinical breakpoints.
[d]Bold formatting indicates that the *spa* type contains *dfrA* and underlining indicates that the *spa* type contains *dfrG*.
[e]Numbers in brackets are numbers of strains with the given *spa* type or harbouring the respective gene.

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

Region	No. of strains	Phenotypic antifolate resistance[a]						TMP resistance gene			
		TMP		SMZ		SXT					
		I	R	I	R	I	R	*dfrA*	*dfrG*	*dfrK*	*dfrB* (F98Y)
Southern Africa[b]	4	0	1 (25%)	1 (25%)	0	1 (25%)	0	0	1	0	0
West Africa[c]	15	0	11 (73%)	7 (47%)	3 (20%)	3 (20%)	3 (20%)	0	11	0	0
East Africa[d]	21	0	10 (48%)	7 (33%)	2 (10%)	2 (10%)	1 (5%)	0	10	0	0
Central Africa[e]	7	0	5 (71%)	1 (14%)	3 (43%)	2 (29%)	1 (14%)	0	5	0	0
Total	47	0	27 (57%)	16 (34%)	8 (17%)	8 (17%)	5 (11%)	0	27	0	0
Pre-travel subjects[f]	81	0	0	5 (6%)[g]	4 (5%)[g]	0	0	0	0	0	0

TMP, trimethoprim; SMZ, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole; I, intermediate resistance; R, resistant.
[a]EUCAST clinical breakpoints were used for the trimethoprim and trimethoprim/sulfamethoxazole resistance phenotypes; CLSI clinical breakpoints were used to define sulfamethoxazole resistance.
[b]Zambia ($n=3$) and South Africa ($n=1$).
[c]Cape Verde ($n=1$), Senegal ($n=2$), Gambia ($n=2$), Guinea-Bissau ($n=1$), Sierra Leone ($n=2$), Ghana ($n=4$), Togo ($n=1$) and Nigeria ($n=2$).
[d]Kenya ($n=8$), Sudan ($n=1$), Rwanda ($n=1$), Uganda ($n=4$), Tanzania ($n=3$), Mozambique ($n=2$) and Malawi ($n=2$).
[e]Cameroon ($n=5$) and Democratic Republic of Congo ($n=2$).
[f]*S. aureus* collected from the nares of 300 subjects during pre-travel consultations.
[g]These nine isolates with altered susceptibility to trimethoprim belong to eight different *spa* types.

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[21,27] based on its isolated occurrence in nosocomial outbreaks of MRSA in London[26] and Chiang Mai, Thailand,[19] and its otherwise sporadic description in staphylococci from animals only.[24,25] Our findings challenge this view and refute the prevailing concept of *dfrB* mutations as the main source of trimethoprim resistance in human *S. aureus*.[16,20,22,27] 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*.[32] Based on similar findings for *dfrA* and *dfrK*,[33] 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[19,26] 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.[4,7,8]

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 overestimation 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[16] accounted for <1% of trimethoprim resistance in sub-Saharan Africa.

For the first time, we detected *dfrK* in *S. aureus* outside of Europe:[17,28] 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,[17,28] occasionally together with *dfrG*,[25] 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,[34] putting our findings in line with existing evidence on the transmission of *S. aureus* harbouring trimethoprim resistance genes between humans and farm animals.[28]

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.[19] 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 *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 *S. aureus* from human infection has major implications for the development of new antifolate antibiotics.[21,23,35] *In vitro* drug testing of candidate compounds is often performed against *S. aureus* carrying *dfrB* mutations or *dfrA* only,[21,23] an approach based on the prevailing view of their key role in determining trimethoprim resistance.[21] Such testing, however, may not warrant activity against *S. aureus* carrying other *dfr* genes. Indeed, one study reported that *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.[35] Together with knowledge on substantial sequence variation between *dfrG* and *dfrA*,[17] 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 *S. aureus* causing human infection reported here, *in vitro* antimicrobial testing of candidate antifolate compounds is recommended to include *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 *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 *S. aureus* on a global scale is needed.[36] 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 *S. aureus* antifolate resistance in sub-Saharan Africa. For example, half of the sample studied from Nigeria consisted of colonizing *S. aureus* from HIV-infected individuals, explaining the comparably higher prevalence of sulfamethoxazole resistance among trimethoprim-resistant *S. aureus* collected there.[9] At the same time, it has to be noted that these heterogeneities do not invalidate our findings on the dominant role of *dfrG* in determining common trimethoprim and trimethoprim/sulfamethoxazole resistance in *S. aureus* on the African continent.

In summary, there is widespread presence of *dfrG* in *S. aureus* from sub-Saharan Africa. Together with this first report on its abundance in *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.[4–7] 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 *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/).

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