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

D. Nurjadi[1], J. Schäfer[2], B. Friedrich-Jänicke[3], A. Mueller[4], A. Neumayr[5,6], A. Calvo-Cano[7], A. Goorhuis[8], N. Molhoek[9], H. Lagler[10], A. Kantele[11,12,13], P. J. J. Van Genderen[9], J. Gascon[7], M. P. Grobusch[8], E. Caumes[14], C. Hatz[5,6,15], R. Fleck[2], F. P. Mockenhaupt[3] and P. Zanger[1,16]

1) *Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg University Hospital, Heidelberg,* 2) *Tropenklinik, Paul-Lechler-Krankenhaus, Tübingen,* 3) *Institute of Tropical Medicine and International Health, Charité – Universitätsmedizin Berlin, Berlin,* 4) *Missionsärztliche Klinik, Würzburg, Germany,* 5) *Swiss Tropical and Public Health Institute,* 6) *University of Basel, Basel, Switzerland,* 7) *ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Barcelona, Spain,* 8) *Centre of Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Academic Medical Centre, University of Amsterdam, Amsterdam,* 9) *Instituut voor Tropische Ziekten, Havenziekenhuis, Rotterdam, The Netherlands,* 10) *Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria,* 11) *Department of Medicine, University of Helsinki,* 12) *Inflammation Centre, Clinic of Infectious Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland,* 13) *Unit of Infectious Diseases, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden,* 14) *Service de Maladies Infectieuses et Tropicales, Groupe Hospitalier Pitié-Salpêtrière, Paris, France,* 15) *Epidemiology, Biostatistics and Prevention Institute, University of Zürich, Zürich, Switzerland and* 16) *Institute of Public Health, Unit of Epidemiology and Biostatistics, Heidelberg University Hospital, Heidelberg, Germany*

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

Original Submission: 19 May 2015; **Revised Submission:** 9 July 2015; **Accepted:** 25 August 2015

Editor: G. Lina

Article published online: XXX

Corresponding author: D. Nurjadi, Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg University Hospital, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany
E-mail: dennis.nurjadi@uni-heidelberg.de

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

http://dx.doi.org/10.1016/j.cmi.2015.08.021

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+ trimethoprim–sulfamethoxazole resistant. On further scrutiny,

Please cite this article in press as: Nurjadi D, et al., Predominance of *dfrG* as determinant of trimethoprim resistance in imported *Staphylococcus aureus*, Clinical Microbiology and Infection (2015), http://dx.doi.org/10.1016/j.cmi.2015.08.021