International Journal of Antimicrobial Agents 50 (2017) 739–741

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Letter to the Editor

First description of *rpsJ* and *mepA* mutations associated with tigecycline resistance in *Staphylococcus aureus* isolated from a cystic fibrosis patient during antibiotic therapy

Sir,

Vancomycin remains the gold-standard treatment for serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections; however, the emergence of strains with reduced vancomycin susceptibility warrants the need for alternative therapies. Tigecycline (TGC) is a broad-spectrum antibiotic that acts as a protein synthesis inhibitor and is active against Gram-positive and Gram-negative organisms, including multidrug-resistant (MDR) bacteria. The US Food and Drug Administration (FDA) approved the use of TGC for the treatment of complicated skin and skin-structure infections, complicated intra-abdominal infections and community-acquired bacterial pneumonia. Multiple studies have described a very low frequency (<0.001%) or complete absence of TGC resistance in *S. aureus* [1,2]. Here we report the first case of TGC-non-susceptible *S. aureus* clinical isolates associated with mutations in the *rpsJ* and *mepA* genes.

In this study, 51 MRSA isolates recovered from respiratory samples of cystic fibrosis (CF) patients attending the Paediatric Respiratory Centre at the Hospital de Niños 'Dr Ricardo Gutiérrez' (Buenos Aires, Argentina) from May 2012 to June 2013 were tested for TGC susceptibility by Etest (bioMérieux). Minimum inhibitory concentration (MIC) results were interpreted based on 2017 European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (resistance, >0.5 μg/mL) (http://www.eucast.org). Quality controls were performed using *S. aureus* ATCC 29213 (susceptible strain, MIC = 0.064 μg/mL) and *S. aureus* 94159m, an in vitro-selected TGC-non-susceptible mutant (MIC = 16 μg/mL) [3].

TGC was active against 98% (50/51) of the isolates studied, with $MIC_{50/90}$ values of 0.064/0.094 μg/mL. The only isolate non-susceptible to TGC (CF39; MIC = 0.75 μg/mL) was recovered from an 8-year-old female CF patient chronically colonised/infected with *S. aureus* and *Escherichia coli* and transiently with *Burkholderia* sp. This patient was not treated with TGC but had received successive treatment with multiple antibiotics, including minocycline. *Staphylococcus aureus* CF38, recovered from the same patient 1 month prior to CF39, was susceptible to TGC (MIC = 0.25 μg/mL). To further investigate the evolution of TGC resistance, six additional isolates recovered from the same patient between September 2014 and November 2015 were also analysed. Fig. 1 describes the antimicrobial therapy received by the patient between 2012 and 2015. Mutations in the *rpsJ* gene (encoding the 30S ribosomal protein S10) and the *mepRAB* operon (multidrug exporter protein MepA), previously described in in vitro-selected mutants [4,5], were screened by sequencing.

All of the isolates were isogenic by pulsed-field gel electrophoresis (PFGE) and were genotypically characterised as ST5-IV-t311-*agr*II. Table 1 shows the MICs for TGC, tetracycline (TET) and minocycline (MIN) as well as the resistance-related mutations for the eight sequential isolates. All isolates, including the TGC-susceptible isolate CF38, had a Lys57Met variation in the ribosomal protein S10 but the TGC-non-susceptible isolates encoded an additional Tyr58Phe variation in this protein. In addition, *mepA* mutations were found in CF187 and in all subsequent isolates. An increase in the TGC MIC was observed in the sequential isolates associated with the amino acid change Leu441Trp in MepA. No mutations were found in the regulatory gene *mepR*. Although TGC-non-susceptible isolates had higher TET and MIN MICs than CF38 and CF186, they were still susceptible to these antibiotics. Mutations in *rpsJ* and *mepRAB* have been described previously in TGC-resistant mutants selected in vitro in *S. aureus* and in other genera

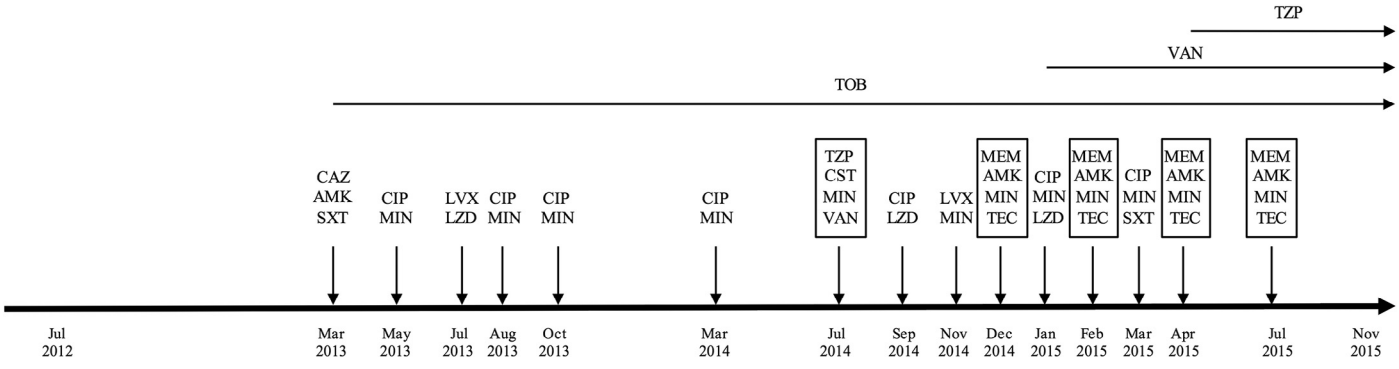

Fig. 1. Antibiotic therapy received by the patient during 2012–2015. Antimicrobial therapy (oral, inhaled and intravenous) is represented above the timeline. Intravenous therapies appear framed and inhaled therapies are illustrated above horizontal arrows (if more than one inhaled antibiotic is prescribed, treatment consists of alternating the antibiotics every month). AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; LVX, levofloxacin; LZD, linezolid; MEM, meropenem; MIN, minocycline; SXT, trimethoprim/sulfamethoxazole; TEC, teicoplanin; TOB, tobramycin; TZP, piperacillin/tazobactam; VAN, vancomycin.

https://doi.org/10.1016/j.ijantimicag.2017.10.003

Table 1
Characteristics of eight temporally-spaced *Staphylococcus aureus* ST5 isolates recovered from an individual cystic fibrosis patient.

Isolation date	CF38 July 2012	CF39 August 2012	CF186 September 2014	CF187 September 2014	CF188 December 2014	CF189 April 2015	CF190 June 2015	CF260 November 2015
TGC MIC (μg/mL) [a]	0.25	0.75	0.5	1	1	1	1.5	2
TET MIC (μg/mL) [b]	2	4	2	4	4	4	4	4
MIN MIC (μg/mL) [b]	1	2	1	2	2	2	2	2
30S ribosomal protein S10 mutation(s)	Lys57Met	Lys57Met; Tyr58Phe	Lys57Met	Lys57Met; Tyr58Phe	Lys57Met; Tyr58Phe	Lys57Met; Tyr58Phe	Lys57Met; Tyr58Phe	Lys57Met; Tyr58Phe
MepA mutation				Leu441Trp	Leu441Trp	Leu441Trp	Leu441Trp	Leu441Trp

TGC, tigecycline; MIC, minimum inhibitory concentration; TET, tetracycline; MIN, minocycline.
[a] The TGC MIC was determined by Etest and the results were interpreted based on 2017 European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (resistance, >0.5 μg/mL).
[b] TET and MIN MICs were determined by broth microdilution method and were interpreted according to 2017 Clinical and Laboratory Standards Institute (CLSI) guidelines.

[4,5]; however, several TGC surveillance studies have previously found no resistance to TGC in *S. aureus* in Argentina [1,2]. Here we describe the first case of TGC-non-susceptible *S. aureus* clinical isolates in Argentina and, to our knowledge, this is the first description of *rpsJ* and *mepA* mutations in an in vivo-selected mutant associated with this phenotype. Isolation of TGC-non-susceptible MRSA from a patient with no previous exposure to this novel antibiotic is highly relevant in the scenario of limited therapeutic options for the treatment of MDR MRSA.

In summary, we describe *S. aureus* sequential isolates recovered from a CF patient in which TGC susceptibility decreased after an extended period of antimicrobial therapy. The non-susceptibility to TGC was associated with mutations in *rpsJ* and *mepA* genes. The mutated positions found in *rpsJ* were concordant with those described by Beabout et al. in in vitro-selected mutants [4]. This study highlights the consequences of multiple antimicrobial treatments during chronic CF infection and the emerging adaptations associated with this phenomenon. More studies are necessary to evaluate the contribution of these mutations to the TGC resistance mechanism and its possible impact on TGC therapeutic efficacy.

Funding: This work was supported in part by grants from Universidad de Buenos Aires, Argentina [UBACYT 20020130100381BA] to MM; Agencia Nacional de Promoción Científica y Tecnológica [PICT 2362] to MM; and a travel award for MSH from Emerging Leaders in the Americas Program (ELAP), Canadian Bureau for International Education (CBIE), Department of Foreign Affairs, Trade and Development (DFATD). MM is a member of 'Carrera del Investigador' from Argentina's National Scientific and Technical Research Council (CONICET). MSH and SDG are doctoral and postdoctoral fellows, respectively, of CONICET.

Competing interests: None declared.

Ethical approval: This study was reviewed and approved by the Research Ethics Committee of the Hospital de Niños 'Dr Ricardo Gutiérrez' (Buenos Aires, Argentina) [CEI N° 16.10].

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M.S. Haim
Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Microbiología, Buenos Aires, Argentina
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

S. Di Gregorio
Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Microbiología, Buenos Aires, Argentina
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

L. Galanternik
Microbiología, Laboratorio Central, Hospital de Niños 'Dr Ricardo Gutiérrez', Buenos Aires, Argentina

S. Lubovich
Centro Respiratorio, Hospital de Niños 'Dr Ricardo Gutiérrez', Buenos Aires, Argentina

M. Vázquez
Microbiología, Laboratorio Central, Hospital de Niños 'Dr Ricardo Gutiérrez', Buenos Aires, Argentina

A. Bharat
R. Zaheer
National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada

G.R. Golding
National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
Department of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, Canada

M. Graham
National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
Department of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, Canada

G. Van Domselaar
National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
Department of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, Canada

S.T. Cardona
Department of Medical Microbiology & Infectious Diseases, University of Manitoba, Winnipeg, Canada

Department of Microbiology, University of Manitoba, Winnipeg, Canada

M. Mollerach *
Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Microbiología, Buenos Aires, Argentina
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

* Corresponding author. Departamento de Microbiología, Inmunología y Biotecnología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956 8th floor, 1113 Buenos Aires, Argentina.
E-mail address: martamollerach@gmail.com (M. Mollerach)

14 August 2017
7 October 2017