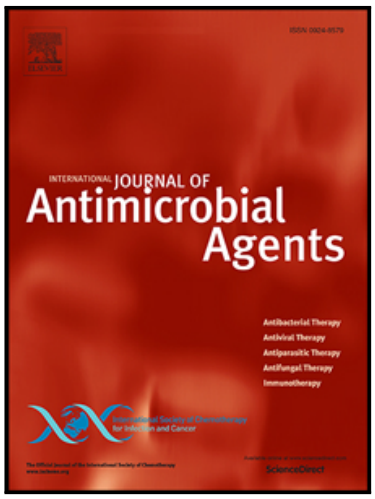

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Successful management with fosfomycin/ceftazidime of an infection caused by multiple highly related subtypes of MDR and XDR KPC-producing *Serratia marcescens*

Cecilia Rodríguez[a,*], Silvina Brengi[b], Mariel Agustina Cáceres[c], Silvana Mochi[c], María Rosa Viñas[b], Constanza Antich Rizza[d], Graciela Merletti[d], Elena Bru[a], José Daniel Assa[d], Raúl Ricardo Raya[a], Daniela Centrón[e]

[a] *Centro de Referencia para Lactobacilos-Consejo Nacional de Investigaciones Científicas y Técnicas (CERELA-CONICET), San Miguel de Tucumán, Argentina*

[b] *Servicio de Enterobacterias, Departamento de Bacteriología, Instituto Nacional de Enfermedades Infecciosas (INEI)-ANLIS "Carlos G. Malbrán", Buenos Aires, Argentina*

[c] *Laboratorio de Bacteriología del Hospital "Ángel C. Padilla", San Miguel de Tucumán, Argentina*

[d] *Laboratorio de Microbiología del Hospital del Niño Jesús, San Miguel de Tucumán, Argentina*

[e] *Instituto de Microbiología y Parasitología Médica, Facultad de Medicina, Universidad de Buenos Aires (IMPaM, UBA-CONICET), Buenos Aires, Argentina*

* Corresponding author. Tel.: +54 0381 4310465; fax: +54 0381 4310465 Ext 124.

E-mail address: mceci_r@hotmail.com (C. Rodríguez)

Sir,

Multidrug (MDR) and extensively drug resistant (XDR) *S. marcescens* infections are increasing in several hospital wards around the world [1-3], probably due to the recent use of colistin to combat Gram-negative carbapenem-resistant isolates [1,3,4]. Here, we describe a particular case of *S. marcescens* infection from a single patient (P1) during four months of hospitalization. Nine *S. marcescens* strains, isolated from consecutive bone and soft tissue samples of P1, were subjected to antibiotype and molecular studies (Table 1). The strains exhibited alternating antimicrobial phenotypes that were either MDR (n = 8) or XDR (showing resistance to all families of antibiotics but two) (n = 1) by the agar disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines and the VITEK®2 semi-automated system (bioMérieux, Marcy-l'Étoile, France) (Tables 1 and S1). Screening methods for the detection of extended-spectrum β-lactamases (ESBLs) and carbapenemases were performed following the guidelines established in CLSI Standards and by the Antimicrobial Subcommittee of SADEBAC-AAM (Argentina, 2007). All strains were resistant to gentamicin, ciprofloxacin and to different combinations of β-lactams (Table 1), and showed to be susceptible *in vitro* to fosfomycin (Table S1). Pulsed-field gel electrophoresis (PFGE) [1,5] showed that the nine *S. marcescens* isolates exhibited five different PFGE subtypes (PFGE profiles XIIa, b, c, d and e) with 97% and 100% of similarity among them (one- to two-band difference) (Table 1). These subtypes were unique compared to PFGE clusters previously found in other hospitals settings in our country, some of them associated with nosocomial outbreaks (Fig. S1) [1,3]. The presence of antimicrobial resistance gene determinants associated with horizontal gene transfer (HGT), and commonly found in Gram-negative MDR-clinical isolates from Argentina, was confirmed by conjugation, PCR amplifications and sequencing

[1,3]. Eight carbapenem-resistant isolates harboured *bla*$_{KPC-2}$ but 7/8 showed unusual susceptibility to ceftazidime (Table 1). Similar to donor cells, the transconjugants harboured the gene resistance markers and showed a ceftazidime-susceptible phenotype (Table S2). This phenotype is unusual for the KPC^{+} nosocomial isolates of our region, despite the fact that KPC-2 has a low efficiency to hydrolyze ceftazidime. Only the XDR SmP7 strain showed a reduced susceptibility pattern to that expected to all the β-lactams for a KPC-positive *Enterobacteriaceae* strain. SmP5 strain did not carry the *bla*$_{KPC-2}$ gene and was susceptible to carbapenems (Table 1); however, this strain carried *bla*$_{CTX-M-2}$ and *bla*$_{SHV-2,}$ genes related to cefepime and ceftazidime resistance, respectively. Isolates SmP1 to SmP4 and SmP7 harboured the *qnrB10* gene (Table 1), which causes a reduction in susceptibility to quinolones (detected by disk diffusion). Also, all strains were found to carry the *bla*$_{CTX-M-2}$ gene in a complex class 1 integron In0::ISCR1::*bla*$_{CTX-M-2}$ which confers resistance to cefotaxime and cefepime, whereas only the SmP4 to SmP7 and SmP9 strains showed the *bla*$_{SHV-2}$ gene (Table 1). The decrease of susceptibility to amikacin, shown by SmP4 to SmP9 strains, could not be related to any of the determinant of resistance assayed. Finally, none of the isolates tested positive for the *bla*$_{GES-like}$, *bla*$_{VEB-like}$, *bla*$_{IMP-like}$, *bla*$_{VIM-like}$, *bla*$_{SPM-1}$ or *aac(6')-Ib* like genes.

The treatment of MDR *S. marcescens* infections often have limited effective options because the natural resistance of this species to several antimicrobial agents, in addition to the acquisition of ESBLs by HGT [1,3]. The observed variation in antibiotic susceptibility profiles and resistance genetic determinants, and the micro-heterogeinity among the *S. marcescens* isolates during the course of the infection of patient P1 was probably a direct consequence of the applied antimicrobial treatment (Table 1). The patient recovered only after the administration of fosfomycin in combination with ceftazidime. This synergistic inhibition has not yet been

documented for this type of infection caused by KPC-harbouring *Enterobacteriaceae* strains. The antimicrobial effect of ceftazidime plus fosfomycin on two *S. marcescens* isolates (XDR-SmP7 and MDR-SmP9) was also studied *in vitro* during kill-curve assays (Fig. S2). Sinergy between the two antibiotics was only observed at 1x MIC, while concentrations above the MIC (2x and 4x MIC) were only bactericidal for both strains (Fig. S2). The mechanism(s) of this beneficial effect might be related to the increase of the bactericidal activity which was 4 h for MDR-SmP9 strain, a time frame which predicts treatment success. This suggest that periods of low concentration of both antibiotics in the bone and soft tissue of patient would allowed a synergistic effect of the combination [6].

To our knowledge, this is the first report of an infection by multiple highly related subtypes of *S. marcescens* $bla_{\text{KPC-2}}$ producers in a single patient. The alarming increase of drug resistance and decreased production of new antibiotics require the evaluation of combinations of existing antibiotics. The successful treatment with a combination of antibiotics (including ceftazidime) described in this study should be further explored in order to provide alternative therapeutic options to the wide dissemination of $bla_{\text{KPC-2}}$ in *Enterobacteriaceae* of nosocomial origin.

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Declarations

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Ethical Approval: Not required.

References

[1] Merkier AK, Rodríguez C, Togneri A, Brengi S, Osuna C, Pichel M, et al. Outbreak of a cluster with epidemic behavior due to *Serratia marcescens* after colistin administration in a hospital setting. J Clin Microbiol 2013;51:2295-302.

[2] Abat C, Desboves G, Olaitan AO, Chaudet H, Roattino N, Fournier PE, et al. Increasing burden of urinary tract infections due to intrinsic colistin-resistant bacteria in hospitals in Marseille, France. Int J Antimicrob Agents 2015;45:144-50.

[3] Rodríguez C, Brengi S, Cáceres MA, Mochi S, Viñas MR, Mereletti G, et al. Polyclonal dissemination of KPC-2 in *Serratia marcescens*, including a clone with epidemic behaviour in the nosocomial niche. Int J Antimicrob Agents 2017;49:657-8.

[4] Arduino SM, Quiroga MP, Ramírez MS, Merkier AK, Errecalde L, Di Martino A, et al. Transposons and integrons in colistin-resistant clones of *Klebsiella pneumoniae* and *Acinetobacter baumannii* with epidemic or sporadic behaviour. J Med Microbiol 2012;61:1417-20.

[5] Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233–9.

[6] Lefort A, Chau F, Lepeule R, Dubée V, Kitzis MD, Dion S, et al. Activity of fosfomycin alone or combined with cefoxitin in vitro and in vivo in a murine model of urinary tract infection due to *Escherichia coli* harbouring CTX-M-15-type extended-spectrum β-lactamase. Int J Antimicrob Agents 2014;43:366-9.

Table 1
Antibiotic treatments administered to patient P1 and microbial and molecular characteristics of *S. marcescens* strains isolated during hospitalization

Treatment date[a]	Treatment /Duration[b]	Surgical toilettes no.[c]	Sampling date[d]	Isolate no.[e]	Antimicrobial resistance pattern[f]	Gene amplification[g]		
						bla$_{KPC}$	*intI1*	*bl*
11/03/13	CZ 1d							
11/04/13	CN+GEN 3d							
11/08/13	CM+GEN 3d							
11/09/13	VAN+IMI 12d	2	11/12/13	SmP1	IMI, MER, ERT, CTX, PTZ, AZT, GEN, CIP^{DS}	+	+	+
			11/18/13	SmP2	IMI, MER, ERT, CTX, PTZ, AZT, GEN, CIP^{DS}	+	+	+
11/21/13	VAN+CIP+TAZ 12d	2	11/22/13	SmP3	IMI, MER, ERT, CTX, PTZ, AZT, GEN, CIP^{DS}	+	+	+
			11/29/13	SmP4	IMI, MER, ERT, CTX, FEP, PTZ, AZT, AK, GEN, CIP^{DS}	+	+	+
12/04/13	CAZ 48d	1	01/20/14	SmP5	CAZ, CTX, FEP, PTZ, AZT, AK, GEN, NAL, CIP, TMS	-	+	+
01/21/14	CIP+COL+IMI 15d	1	01/27/14	SmP6	IMI, MER, ERT, CTX, FEP, PTZ, AZT, AK, GEN, CIP^{DS}	+	+	+
02/05/14	CIP+CAZ+TIG 30d	2	02/11/14	SmP7	IMI, MER, ERT, CAZ, CTX, FEP, PTZ, AZT, AK, GEN, NAL, CIP, TMS	+	+	+
			02/25/14	SmP8	IMI, MER, ERT, CTX, PTZ, AZT, AK, GEN, CIP, TMS	+	+	+
03/08/14	CAZ+FOS 15d	1	03/11/14	SmP9	IMI, MER, ERT, CTX, PTZ, AZT, AK, GEN, CIP, TMS	+	+	+
03/24/14	Patient recovered							

[a] Start date of the antibiotic treatment (mo/day/yr).
[b] Duration of the antibiotic treatment received by patient P1. d = day. Cz, cefazolin (2g, oral), surgery prophylactic antibiotic treatment. CN, cephalexin (2 g/24h, oral) plus GEN, gentamicin (160 mg/24 h, intramuscular, IM). CM, clindamycin (600 mg/6 h, intravenous, IV) plus GEN (160 mg/24 h, IM). VAN, vancomycin (1 g/12 h, IV) plus IMI, imipenem (500 mg/6 h, IV), empiric treatment. Beginning of successive surgical toilettes with the following schemes: VAN (1

g/12 h, IV) plus CIP, ciprofloxacin (200 mg/12h, IV) plus TAZ, tazobactam (4,5 g/6h, IV). CAZ, ceftazidime (2 g/24h, IV). CIP (200 mg/12h, IV) plus COL, colistin (300 mg/24h, IV) plus IMI (500 mg/6 h, IV). CIP (200 mg/12h, IV) plus CAZ (2 g/24h, IV) plus TIG, tigecycline (50 mg/12h, IV). CAZ (2 g/24h, IV) plus FOS, fosfomycin (10 g/24h, IV).

[c] Number of surgical toilettes performed during antibiotic treatment.

[d] Isolation dates of *S. marcescens* strains from patient P1.

[e] Nomenclature of S. *marcescens* strains isolated from patient P1 (from SmP1 to SmP9).

[f] Antimicrobial agents tested were: IMI, imipenem; MER, meropenem; ERT, ertapenem; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; PTZ, piperacillin/tazobactam; AZT, aztreonam; AK, amikacin; GEN, gentamicin; NAL, nalidixic acid; CIP, ciprofloxacin; TMS, trimethoprim/sulfamethoxazole; TIG, tigecycline; FOS, fosfomycine. DS, decrease susceptibility.

[g] - and +, negative and positive, respectively, for gene amplification by PCR by specific primers [1,3].

[h] Genotype determined by pulse field gel electrophoresis (PFGE) patterns arbitrarily named with a Roman number [1].