International Journal of Antimicrobial Agents 49 (2017) 657–658

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

Letter to the Editor

Polyclonal dissemination of KPC-2 in *Serratia marcescens*, including a clone with epidemic behaviour in the nosocomial niche

Sir,

Serratia marcescens is a nosocomial pathogen frequently isolated from outbreaks with high morbidity and mortality [1–3]. Several nosocomial outbreaks have been described caused by the dispersion of epidemic clones carrying class 1 integrons [1–3], some of which also harbour *Klebsiella pneumoniae* carbapenemase-2 (KPC-2) in carbapenem-resistant *S. marcescens* nosocomial isolates [3,4], rendering phenotypes of extreme antimicrobial resistance. Previously, we have identified multidrug-resistant (MDR) *S. marcescens* strains belonging to epidemic cluster IX circulating since 2002 in Argentinian nosocomial isolates [2]. This cluster was susceptible to carbapenems and harboured class 1 integrons. Later, a significant increase in the frequency of carbapenem-resistant *S. marcescens* nosocomial isolates (19.5/11.9 absolute/expected annual frequency) was identified in a hospital of northern Argentina (H1) between 2013 and 2014.

A total of 60 *S. marcescens* strains isolated from different patients hospitalised in several wards of H1 were registered during the period August 2013–2014, of which 28 (46.7%) were carbapenem-resistant (SmCR). Of these 28 non-epidemiologically related SmCR isolates, 20 were subjected to microbiological and molecular analysis (Fig. 1). All isolates showed a MDR profile by the agar disk diffusion method performed according to the Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines and using a VITEK®2 semi-automated system (bioMérieux, Marcy-l'Étoile, France). Screening methods for the detection of extended-spectrum β-lactamases (ESBLs) and carbapenemases were performed in accordance with CLSI standards and the recommendations of the Antimicrobial Subcommittee of the Sociedad Argentina de Bacteriología, Micología y Parasitología Clínica (SADEBAC-AAM, 2007). All isolates were shown to harbour bla_{KPC-2} by PCR amplification with specific primers (forward, 5′-TGTCACTGTATCGCCGTC′-3; and reverse, 5′-GCTTGTCATCCTTGTTAG′-3) and sequencing. Genotyping was carried out using pulsed-field gel electrophoresis (PFGE) as previously described [2]. The 20 non-epidemiologically related SmCR isolates from this study were compared with 55 other isolates available in the PFGE National Database of *S. marcescens* genetic subtypes corresponding to nosocomial infections and sporadic cases from 12 different Argentinian hospitals [2], which rendered a total of 44 different subtypes of *S. marcescens* identified in our country (data not shown). Twelve of these subtypes included the 20 SmCR isolates from the current study, in nine defined clusters as shown in Fig. 1. Six different genetic subtypes of *S. marcescens* from the PFGE National Database, three of them KPC-producing isolates (419, 804 and 889) are also shown (Fig. 1). Isolate SmCR 371 from the present study belongs to cluster IX, which was previously isolated from outbreaks in several hospitals in Argentina [2], exhibiting epidemic behaviour and susceptibility to carbapenems. SmCR strains belonging to cluster I corresponded to the prevalent pattern ($n = 9$ isolates) detected from September 2013 to February 2014, mainly in the intensive care area, suggesting a common source of infection. SmCR strains belonging to clusters III, VI and VIII were shown to be genetically related to others isolates of the PFGE National Database recovered from the same hospital H1 (62, 197 and 419) but with different antimicrobial resistance mechanisms, probably due to lateral antimicrobial resistance gene transfer events (Fig. 1). The remaining SmCR clusters identified in this work were not detected previously in other *S. marcescens* strains from the PFGE National Database of Argentina (Fig. 1).

PCR amplification and sequencing of the most prevalent antimicrobial resistance determinants were performed: integron integrase genes (*intI1*); $bla_{CTX-M-2}$, $bla_{SHV-like}$, bla_{PER-2}, bla_{NDM}, $bla_{GES-like}$, $bla_{VEB-like}$, $bla_{KPC-like}$, $bla_{VIM-like}$, $bla_{IMP-like}$, $bla_{SME-like}$ and $bla_{SPM-like}$ [2,5]; and complex class 1 integrons [2]. Thirteen isolates carried *intI1*, including seven of nine isolates corresponding to prevalent cluster I as well as the epidemic clone previously described in Argentina belonging to cluster IX (Fig. 1). Also, the coexistence of other β-lactamases such as bla_{SHV-2} and $bla_{CTX-M-2}$ in unrelated clones (SmCR 1164, SmCR 979, SmCR 1013, SmCR 371, SmCR 917, SmCR 709 and SmCR 495) was detected (Fig. 1). None of the studied isolates harboured bla_{PER-2}, bla_{NDM}, $bla_{GES-like}$, $bla_{VEB-like}$, $bla_{VIM-like}$, $bla_{IMP-like}$, $bla_{SME-like}$ or $bla_{SPM-like}$ genes.

Recently, an increase in *S. marcescens* infections has been observed in hospital settings from Argentina and France related to previous colistin administration [2,6]. In this regard, use of colistin for the treatment of carbapenem-resistant infections should be reconsidered, as it not only increases the incidence of infections by *S. marcescens* [2,6] but also contributes to the selection of different clones able to acquire, maintain and disseminate bla_{KPC-2} in this species (Fig. 1).

This study also shows both the acquisition of bla_{KPC-2} by *S. marcescens* in an epidemic cluster associated with outbreaks circulating for more than 15 years in our country such as cluster IX [2] as well as the clonal variability of KPC-producing *S. marcescens* strains. This competence suggests a genomic plasticity of *S. marcescens* species for its adaptation to multidrug and extreme drug resistance within the nosocomial niche.

Acknowledgements

The authors are grateful to L. Acuña, A. Amilaga, N. Biñon, G. Cabrera, A. Recupero and G. Sanchez (Hospital 'Ángel C. Padilla', San Miguel de Tucumán, Tucumán, Argentina) for technical assistance. CR, RRR and DC are members of the Carrera del Investigador Cientifico (CONICET).

Funding: This work was partially supported by PICT 0014-2012 and UBACYT Programación 2014–2017 given to DC; and PICT 2014-3428 given to CR.

Competing interests: None declared.

Ethical approval: Not required.

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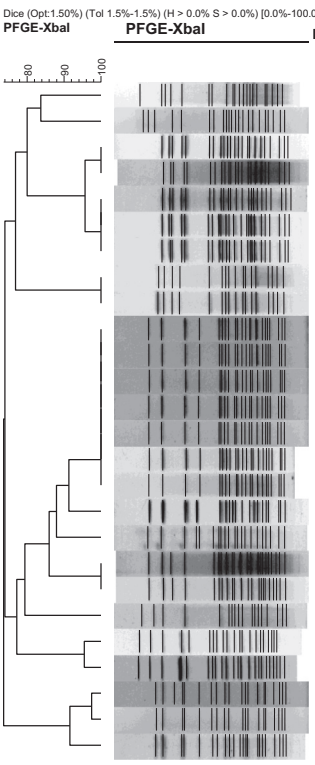
http://dx.doi.org/10.1016/j.ijantimicag.2017.03.003



Isolate [a]	Date of isolate (yr-mo-day)	Hospital/ Wards [b]	Isolate source [c]	PFGE subtype [d]	Gene amplification [e] *intI*	*bla*KPC	*bla*CTX-M-2	*bla*SHV-2	Antimicrobial sensitivity pattern [f]
SmCR 451	2014-08-08	H1/ICA	BF	VII	-	+	-	-	CAZ, FEP, AZT, AK, GEN, TIG, TMS
SmCR 917	2014-02-22	H1/SW	CAT	II	-	+	-	+	NAL, CIP, TIG, TMS
419 ND	2014-02-11	H1/TW	ST	VI	+	+	+	+	TIG, FOS
SmCR 709	2014-01-18	H1/ICA	CAT	VI	+	+	+	-	AK, TIG
SmCR 1363	2013-11-27	H1/TW	ST	VI	-	+	-	-	CAZ, AK, TIG, TMS
804 ND	2013-11-17	H1/TW	ST	VI	+	+	+	-	CAZ, ERT, AK, NAL, TMS, FOS
889 ND	2013-11-19	H1/TW	ST	VI	+	+	+	-	CAZ, AK, NAL, TMS, FOS
SmCR 748	2014-02-19	H1/SW	BD	VIII	+	+	-	-	PTZ, FEP, ERT, GEN, CIP, TIG
62 ND	2014-01-02	H1/CW	ST	VIII	-	-	-	-	CAZ,CTX,FEP,PTZ,AZT,IMI,MER,ERT,AK,GEN,NAL,CIP,TIG,TMS
SmCR 1373	2013-09-30	H1/SR	TA	I	+	+	-	-	GEN, TIG, TMS
SmCR 375	2013-10-07	H1/ICA	BF	I	+	+	-	-	AK, GEN. NAL, CIP, TIG, TMS
SmCR 720	2013-11-14	H1/EM	BF	I	+	+	-	-	FEP, AK, GEN. NAL, CIP, TIG, TMS
SmCR 1164	2013-11-22	H1/CW	BF	I	-	+	-	+	AK, GENT, NAL, CIP, TIG, TMS
SmCR 1150	2013-11-27	H1/ICA	BF	I	+	+	-	-	AK, GEN, NAL, CIP, TIG
SmCR 979	2014-02-24	H1/ICA	BD	I	+	+	+	-	TIG, TMS
SmCR 1013	2014-02-02	H1/CW	ST	I	+	+	-	+	GENT, NAL, CIP, TIG, TMS
SmCR 562	2013-11-12	H1/ICA	BF	I	-	+	-	-	CAZ, AK, GEN, NAL, CIP, TIG, TMS
SmCR 55	2013-10-01	H1/ICA	BD	I	+	+	-	-	CAZ, AK,GEN,NAL, CIP,TIG, TMS
SmCR 1358	2013-11-27	H1/SW	NOD	IV	+	+	-	-	CAZ, FEP, TIG, TMS
SmCR 483	2014-01-10	H1/NW	TA	IV	+	+	-	-	AK, GEN, CIP, TIG, TMS
SmCR 556	2014-07-15	H1/ICA	BF	V	-	+	-	-	PTZ, CAZ, CTX, FEP, AK, GEN, NAL, CIP, TIG, TMS
SmCR 371	2013-09-07	H1/ICA	CAT	IX	+	+	+	-	AK, NAL, TMS
13006 ND	2007-12-23	H2	BD	IX	+	-	+	-	IMI, MER
SmCR 495	2013-11-11	H1/CW	ST	III	+	+	+	-	FEP, GEN, TIG, TMS
SmCR 37	2014-01-02	H1/ICA	BF	III	-	+	-	-	CAZ, FEP, GENT, TIG, TMS
197 ND	2013-07-05	H1/ICA	UR	III	+	-	+	-	MER, IMI, TAZ, ERT

Fig. 1. *Xba*I pulsed-field gel electrophoresis (PFGE) dendrogram and microbiological and molecular features of *Serratia marcescens* strains from this study. [a] SmCR are *S. marcescens* carbapenem-resistant isolates from hospital H1; ND are different genetic subtypes of *S. marcescens* from the PFGE National Database. Strain 13006 was isolated from outbreak [2] and strains 419, 804, 889, 62 and 197 were sporadic strains. [b] All 20 SmCR strains from this study were isolated from infected patients from different wards of the hospital: ICA, intensive care area; SW, surgery ward; TW, traumatology ward; CW, clinical ward; SR, shock room; EM, emergency; NW, neurology ward. [c] BF, bronchoalveolar fluid; CAT, catheter; ST, soft tissue; BD, blood; TA, tracheal aspirate; NOD, nodule; UR, urine. [d] The cut-off value for cluster delineation was >91% similarity. Pulsed-field gel electrophoresis (PFGE) patterns were arbitrarily named with a roman number. [e] Negative and positive for gene amplification by PCR. [f] All isolates were resistant to the remaining antibiotic agents. CAZ, ceftazidime; FEP, cefepime; AZT, aztreonam; AK, amikacin; GEN, gentamicin; TIG, tigecycline; TMS, trimethoprim-sulfamethoxazole; NAL, nalidixic acid; CIP, ciprofloxacin; FOS, fosfomycin; ERT, ertapenem; PTZ, piperacillin/tazobactam; CTX, cefotaxime; IMI, imipenem; MER, meropenem.

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2 January 2017
11 March 2017
http://dx.doi.org/10.1016/j.ijantimicag.2017.03.003