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Letter to the Editor

Carbapenem-hydrolysing β-lactamase KPC-2 in *Klebsiella pneumoniae* isolated in Ecuadorian hospitals

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Sir,

The carbapenems are regarded as the preferential therapeutic option for treatment of serious healthcare-associated infections with multidrug-resistant Gram-negative bacteria. However, carbapenem resistance has been increasingly reported worldwide. This is largely due to the emergence and spread of *Klebsiella pneumoniae* carbapenemase (KPC) [1]. In this paper, we report the first genotypification of KPC-2-producing *K. pneumoniae* isolates in Ecuador.

The first case in Ecuador of KPC-2 β-lactamase in a carbapenem-resistant *K. pneumoniae* isolate occurred in October 2010 [2]. This report came from Azogues, a city located in the centre of the country. According to the authors, one carbapenem-resistant *K. pneumoniae* strain harbouring the bla_{KPC-2} gene was isolated from a subgaleal abscess following meningioma surgery. During the years 2010–2012, 22 carbapenem-resistant *K. pneumoniae* were isolated from distinct patients hospitalised in intensive care units (ICU) of tertiary hospitals located in Quito, Azogues, Guayaquil and Cuenca. These bacteria were isolated from blood (four isolates), urine (one isolate), the respiratory tract (seven isolates), skin and soft tissue (nine isolates) and pancreatic fluid (one isolate) (Table 1). The antimicrobial susceptibility profile of the isolates was determined using VITEK® 2 (bioMérieux, Lyon, France) for imipenem and ertapenem according to Clinical and Laboratory Standards Institute (CLSI) guidelines [3], and using M.I.C. Evaluator™ (M.I.C.E.) strips (Oxoid Ltd., Basingstoke, UK) for tigecycline and GNXF Sensititre® (TREK Diagnostic Systems, Westlake, OH) for colistin according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [4]. Primers bla_{KPC} Forward (5′-CGGAACCATTCGC-TAAACTC-3′) and bla_{KPC} Reverse (5′-GGCCTCGCTGTRCTTGTCAT-3′) [2] were used to amplify the bla_{KPC} gene (738 bp), and amplicons were sequenced by Macrogen (Seoul, South Korea). The presence of additional β-lactamase genes bla_{GES} [5], bla_{CTX} [6], bla_{TEM}, bla_{VIM} and bla_{IMP} [7] was evaluated by PCR amplification. Pulsed-field gel electrophoresis (PFGE) was performed using *Xba*I. An unweighted pair-group method with arithmetic mean (UPGMA) dendrogram based on PFGE was constructed using Dice coefficients with BioNumerics software (Applied Maths, Kortrijk, Belgium).

All *K. pneumoniae* clinical isolates showed resistance to broad-spectrum cephalosporins (data not shown) and carbapenems (Table 1). The PFGE dendrogram grouped the carbapenem-resistant *K. pneumoniae* isolates into four clades using 80% similarity, each represented by isolates from different healthcare facilities; in addition, several samples were not part of any group (Table 1). These patterns suggest that more than one clone is circulating in Ecuador (Table 1).

Presence of the bla_{KPC-2} gene was confirmed in all isolates by sequencing results. All of the isolates also carried at least one bla_{CTX-M} gene (groups 1, 2 and 9). Group 1 was the most commonly found; however, in one isolate (CB-QCA 3436) all three groups were detected (Table 1). Furthermore, β-lactamase genes bla_{TEM} (65.2%; 15/23), bla_{GES} (17.4%; 4/23) and bla_{VIM} (13.0%; 3/23) were detected. These results are comparable with other studies reporting KPC-producing isolates accumulating β-lactam resistance enzymes TEM-1, CTX-M-2 and SHV-11 [8].

The report of KPC-2-producing isolates is very worrisome since these isolates often carry other genes that confer resistance to β-lactam agents and other antimicrobials. This attribute limits the therapeutic options available for treatment of serious infections, now essentially restricted to tigecycline and polymyxins. Nevertheless, three isolates showed tigecycline

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Table 1
β-Lactamase genes and genotypification of carbapenem-resistant *Klebsiella pneumoniae* isolates as well as susceptibility profiles to tigecycline (TGC), colistin (COL), imipenem (IPM) and ertapenem (ERT).

Code	City	Source	Patient status	β-Lactamase genes						PFGE clades (80% similarity)[a]	MIC (mg/L)			
				bla_{TEM}	bla_{CTX} group	bla_{KPC-2}	bla_{IMP}	bla_{VIM}	bla_{GES}		TGC[b]	COL[c]	IPM[d]	ERT[d]
CB-QCA 3478	Quito	Skin and soft tissue	N/D	+	1	+	–	+	–	I	8	1	≥16	≥8
CB-QCA 3482	Quito	Respiratory tract	N/D	–	1	+	–	–	–	I	4	1	≥16	≥8
CB-QCA 3467	Quito	Respiratory tract	Dead	–	9	+	–	–	+	II	1	0.5	≥16	≥8
CB-QCA 3468	Quito	Skin and soft tissue	N/D	–	2	+	–	–	+	II	1	0.5	≥16	≥8
CB-QCA 3477	Quito	Skin and soft tissue	N/D	+	2	+	–	+	–	II	1	0.5	≥16	≥8
CB-QCA 3466	Cuenca	Respiratory tract	Dead	+	2, 1	+	–	–	–	III	1	0.5	8	≥8
CB-QCA 3480	Quito	Blood	Dead	+	1	+	–	–	–	III	1	0.5	≥16	≥8
CB-QCA 3483	Quito	Skin and soft tissue	N/D	–	1	+	–	–	–	III	1	0.5	≥16	≥8
CB-QCA 3484	Quito	Blood	Dead	+	1	+	–	–	–	III	1	0.5	≥16	≥8
CB-QCA 3500	Guayaquil	Respiratory tract	N/D	+	2, 1	+	–	–	–	III	1	0.5	8	≥8
CB-QCA 3435[e]	Azogues	Subgaleal abscess	Dead	–	1	+	–	–	–	IV	1	0.5	8	≥8
CB-QCA 3439	Azogues	Respiratory tract	Dead	+	1	+	–	–	–	IV	1	0.5	≥16	≥8
CB-QCA 3464	Guayaquil	Respiratory tract	Dead	–	1	+	–	–	–	NG	1	0.5	≥16	≥8
CB-QCA 3481	Quito	Skin and soft tissue	N/D	–	1	+	–	+	–	NG	4	1	≥16	≥8
CB-QCA 3441	Quito	Skin and soft tissue	N/D	+	9	+	–	–	–	NG	2	1	<1	≥8
CB-QCA 3437	Cuenca	Urine	N/D	+	9, 1	+	–	–	–	NG	1	1	8	≥8
CB-QCA 3440	Quito	Respiratory tract	Dead	+	1	+	–	–	–	NG	1	1	<1	≥8
CB-QCA 3461	Quito	Blood	Dead	–	1	+	–	–	–	NG	1	1	≥16	≥8
CB-QCA 3462	Guayaquil	Pancreatic fluid	N/D	+	1	+	–	–	+	NG	1	1	≥16	≥8
CB-QCA 3436	Guayaquil	Blood	Dead	+	2, 9, 1	+	–	–	–	NG	1	1	2	≥8
CB-QCA 3450	Guayaquil	Skin and soft tissue	Dead	+	1	+	–	–	–	NG	0.5	0.5	≥16	≥8
CB-QCA 3442	Quito	Skin and soft tissue	N/D	+	2	+	–	–	+	NG	1	0.25	4	4
CB-QCA 3438	Azogues	Skin and soft tissue	Dead	+	1	+	–	–	–	NG	1	1	≥16	≥8

MIC, minimum inhibitory concentration; N/D, no data available; PFGE, pulsed-field gel electrophoresis.
[a] NG, not grouped (strains that did not group in any clade in the UPGMA tree using 80% similarity).
[b] M.I.C. Evaluator™ (M.I.C.E.) strips (Oxoid Ltd., Basingstoke, UK).
[c] GNXF Sensititre® (TREK Diagnostic Systems, Westlake, OH).
[d] VITEK® 2 (bioMérieux, Lyon, France).
[e] Strain CB-QCA 3435 correspond to the first report of *K. pneumoniae* producing carbapenemase KPC-2 in Ecuador [2].

resistance (according to EUCAST, 2013) (Table 1). In addition, the detection of such isolates by clinical laboratories might be difficult when current standard antimicrobial susceptibility methods are employed. Moreover, bla_{KPC-2} is plasmid-borne, making its dissemination easier, especially when carried by *K. pneumoniae*, an organism notorious for its ability to accumulate and transfer resistance determinants.

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Competing interests

None declared.

Ethical approval

Not required.

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