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Short communication

Performance of a PCR assay for the rapid identification of the *Klebsiella pneumoniae* ST258 epidemic clone in Latin American clinical isolates

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ABSTRACT

The worldwide dissemination of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* ST258 demands a rapid PCR-based typing method to detect unique genes of the ST258 clone. This study evaluates a PCR developed by Adler et al. (2014) for the detection of ST258 in *K. pneumoniae* clinical isolates centered on the identification of the *pilv-I* and *prp* genes. We tested 143 clinical isolates from Argentina ($n = 109$), Chile ($n = 1$), Colombia ($n = 1$), Costa Rica ($n = 2$), Ecuador ($n = 5$), El Salvador ($n = 2$), Nicaragua ($n = 5$), Panamá ($n = 2$), Paraguay ($n = 2$), Perú ($n = 3$) and Trinidad and Tobago ($n = 11$) recovered from 2006 to 2015. bla_{KPC}, *pilv-I* and *prp* genes were detected by PCR and sequenced by standard procedures. ST258 and non-ST258 were defined by PFGE and/or MLST. Isolates were grouped according to PFGE patterns: 58 were compatible with ST258 (group 1) and 85 with non-ST258 (group 2). MLST study was done on an arbitrary selection of isolates. The *pilv-I* gene was present only in ST258 isolates, regardless of the presence of the bla_{KPC} gene. Results for the *prp* gene were variable. Its presence did not define ST258. The *pilv-I* PCR had a sensitivity and specificity of 100%, respectively, for the detection of ST258 in the isolates under investigation. Given our findings, the *pilv-I* PCR could replace more time and resource consuming methods, allowing for more rapid detection of the circulating high risk *K. pneumoniae* clone ST258 in Latin American (LA) countries.

The worldwide dissemination of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* ST258 demands the development of a PCR-based rapid typing method to detect genes unique for the ST258 clone (Adler et al., 2014). The study presented by Adler et al., highlighted two likely gene candidates: *pilv-I* and *prp*. The former codes for a protein of unknown function believed to be unique for the ST258 clone. The latter is a phage related protein, with a non-exclusive relationship to the ST258 clone, which was used additionally in high prevalence settings to enhance detection sensitivity for the ST258 clone (Adler et al., 2014). One of the limitations of this study was the low number of isolates from LA, where the actual prevalence of ST258 is unknown. Regardless of this, the spread of ST258 has been documented in several LA countries like Colombia (Lopez et al., 2011), Argentina (Gomez et al., 2011) and Brazil (Andrade et al., 2011) among others, representing a significant problem for health institutions.

This study aims to evaluate the PCR protocol proposed by Adler et al. using isolates referred to the Regional Reference Laboratory in Antimicrobial Resistance (LRR).

The countries belonging to the Latin American Network for Monitoring Antimicrobial Resistance (ReLAVRA), refer representative carbapenem non-susceptible *K. pneumoniae* isolates to the LRR in Argentina for molecular characterization. In this context, the scheme under investigation was evaluated on a total of 143 non-duplicate and patient unique *K. pneumoniae* clinical isolates, (117 KPC producers and 26 non-KPC producers). The isolates evaluated were primarily from Argentina ($n = 109$) and 10 LA countries ($n = 34$): Chile (1), Colombia (1), Costa Rica (2), Ecuador (5), El Salvador (2), Nicaragua (5), Panamá (2), Paraguay (2), Perú (3) and Trinidad and Tobago (11) from the period 2006 to 2015. PCR assays were performed to detect the following genes: bla_{KPC} (Gomez et al., 2011), *pilv-I* (5′-TGATGCTGATGGCAGACTGA and 5′-TGTAGTCACACCCTGCCCA, product size 320 bp), *prp* (primers 5′-GTGCACGATACAGGCTTCAA and 5′-GAGGTCCTACAATCGGAGCA, product size 544 bp) (Adler et al., 2014) and CTXM-15 (Blanco et al., 2009). Sequencing was performed to all KPC isolates to detect the bla_{KPC} allele, and to the first amplicons obtained from *pilv-I* and *prp* genes, to confirm the correct PCR amplification. Pulsed field gel electrophoresis (PFGE) of Xba-1 digested genomic DNA was performed for all the

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Table 1
PCR detection of *pilv-l* and *prp* genes in *K. pneumoniae* ST258 and non-ST258 isolates.

ST	n	bla_{KPC}	*pilv*-l (+)	*prp* (+)
ST258	55	+	55	25
	3	–	3	3
Non-ST258	62	+	0	7
	23	–	0	1

+, positive; −, negative.

isolates. They were then analyzed according to Tenover's criteria (Tenover et al., 1995). The isolates were first grouped according to PFGE patterns: 58 were compatible with ST258 (group 1) and 85 with non-ST258 (group 2). Arbitrary selection of isolates were chosen for MLST study considering hospital, date of isolation, country and PFGE pattern, using the *K. pneumoniae* MLST website (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html).

Fifteen isolates from group 1 were confirmed as ST258. Eighteen isolates from group 2 were confirmed as ST14 ($n = 1$), ST20 ($n = 1$), ST25 ($n = 1$), ST34 ($n = 1$), ST37 ($n = 1$), ST101 ($n = 2$), ST147 ($n = 1$), ST265 ($n = 1$), ST476 ($n = 1$), ST526 ($n = 1$) and two single locus variants of ST258: ST11 ($n = 6$) and ST437 ($n = 1$). These sequence types were detected in the Argentinian isolates, except for ST101 from Chile and ST437 from Nicaragua. Isolates from Argentina, Chile, Colombia, Costa Rica, Ecuador, Nicaragua and Paraguay harbored bla_{KPC-2}. One isolate from El Salvador harbored bla_{KPC-2} and the other bla_{KPC-3} (both *pilv-l* positive). Two Peruvian isolates harbored bla_{KPC-2} and the third one bla_{KPC-3}. The isolates from Panama and Trinidad and Tobago harbored bla_{KPC-3}.

The *pilv-l* gene was present only in ST258 isolates, regardless of the presence of bla_{KPC} (Table 1). Three ST258 isolates from Argentina were positive for *pilv-l* but negative for bla_{KPC}. They carried $bla_{CTX-M-15}$, confirming the circulation of ST258 without bla_{KPC}. All *pilv-l* negative isolates were non-ST258.

Results for the *prp* gene were variable and did not define the clonal group as ST258 or non-ST258. In contrast, the application of the *pilv-l* PCR resulted in a sensitivity and specificity of 100% for the detection of the *K. pneumoniae* ST258 clone in the isolates tested.

In summary, our results demonstrate that in the isolates evaluated, the detection of *pilv-l* alone is enough to discriminate *K. pneumoniae* ST258 from other STs. Amplification of *prp* did not enhance the sensitivity of the method. Therefore, the pilv-l PCR method could be used in Latin America as a rapid and low cost screening method for the detection of the hyperepidemic *K. pneumoniae* ST258 clone.

Conflict of interest

Nothing to declare.

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