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Brief Report

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In silico analysis of transferable QepA variants and related chromosomal efflux pumps

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ABSTRACT

Objectives. The present study aimed to detect the presence of undescribed QepA variants in GenBank records.

Material and methods. The DNA and amino acid sequences of QepA1 were compared with what is present in GenBank. Only annealings with a >80% identity were considered. No synthetic or partial sequences were included in the analyses.

Results. The results showed the presence of 10 different QepA variants, 6 of them which were previously non-designated as specific allelic variants. In addition, high identity levels with chromosomal MSF efflux pumps belonging to microorganisms of the *Pseudorhodoferax* genus and other *Comamonadaceae* were detected.

Conclusions. The presence of undescribed QepA variants in GenBank is reported and the presence of related sequences among members of *Burkholderiales* order is described.

Keywords: QepA; *Comamonadaceae*; transferable mechanisms of quinolone resistance.

Análisis *in silico* de variantes transferibles de QepA y de bombas cromosomales de eflujo relacionadas

RESUMEN

Objetivos. El objetivo del presente estudio fue detectar la presencia en GenBank de variantes de QepA no descritas.

Materiales y métodos. Las secuencias de ADN y aminoácidos de QepA1 se compararon en GenBank. Sólo se consideraron las identidades >80%. Las secuencias sintéticas o parciales no se incluyeron en los análisis.

Resultados. Los resultados mostraron la presencia de 10 variantes de QepA, 6 de ellas sin designación alélica. Adicionalmente se observaron elevados niveles de identidad con bombas MSF cromosomales del genero *Pseudorhodoferax* y de otras *Comamonadaceae*.

Conclusiones. Se reporta la presencia en GenBank de variantes no descritas de QepA, así como la presencia en miembros del orden *Burkholderiales* de secuencias relacionadas.

Palabras Clave: QepA; *Comamonadaceae*; Mecanismos Transferibles de Resistencia a Quinolonas.

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INTRODUCTION

The efflux pump QepA was first described in 2007 [1,2]. This efflux pump may extrude several quinolones from bacteria, thereby conferring low levels of resistance to these antimicrobial agents and favoring the selection of new alterations able to induce full quinolone resistance [1-3]. In 2008 a new Qep variant differing in 2 amino acid positions was described and named as QepA2 [4]. No further QepA variant was reported until 2015, when QepA3 was described [5]. Finally, in 2017 two independent groups described the last QepA variant with the allelic designation QepA4 [6,7].

A recent study on Qnr (other transferable mechanisms of quinolone resistance), reported the presence of both a high number of erroneous attributions, as well as a series of undescribed alleles, mostly related to the increasing number of sequenced genomes [8].

Therefore, the present study aimed to review the different published QepA variants, determining the presence of hidden transferable QepA variants in GenBank, as well extending the search to closely related chromosome-encoded sequences.

MATERIAL AND METHODS

The QepA1 amino acid (GenBank access: BAF63420) and DNA (GenBank access: AB263754) type sequence were compared with what was present in GenBank on July 29, 2018 using the Blast tool. All *qepA* sequences detected amongst microorganisms non-suggestive of being the *qepA* gene ancestor were classified as transferable, irrespective of their chromosome or plasmid encoding loci. Only full sequences were included in the analysis. In all cases it was confirmed whether the sequence was recorded as "QepA", "quinolone efflux pump" or similar references. No annealing with <85% of identity was considered. In addition, a revision of PubMed literature was done to determine both the presence of published reports of the different QepA variants, as well as to identify the microorganisms in which they have most frequently been reported.

The QepA variants recorded in GenBank for which no allelic numeration has been previously established has been designated in this study according: 1) Publishing date of the article in which the sequence was reported (even if the GenBank access provided belonged to the whole bacterial genome); 2) When no article was found the numeration was done in accordance with the oldest GeneBank access record.

RESULTS AND DISCUSSION

Forty-nine sequences fulfilled the selection criteria; 45 were recorded with amino acid data, and of these, 4 QepA sequences had been published with an allelic numeric reference (QepA1 to QepA4) [1,2,4-7] while 3 others had been published without allelic identification [9-11]. The 4 *qepA* DNA sequences without protein translation were identified as *qepA1* (3 sequences) and *qepA4*. Overall, 10 different allelic variants were identified all of which possessed identity levels higher than 99%, differing by a maximum of 5 amino acid residues (table 1). The results showed that most of the studies reporting the presence of QepA were performed in Enterobacteriaceae but also in other Gram-negative bacterial families such as Pseudomonadaceae or Moraxellaceae [12,13] (table 2).

Most of the studies in this field have been focused on the detection of the presence/absence of the *qepA* gene, without analyzing the presence of specific *qepA* alleles. This approach provides information about the presence of a specific mechanism of quinolone resistance. Nonetheless, slightly divergent QepA variants might present differences in substrate specificities leading to increased or decreased affinities or expanding/limiting the spectrum of substrates, as has been described in other efflux pumps [14-16]. Indeed, although it is largely accepted a positive amplification of a *qepA* gene fragment to classify a microorganism as $QepA^{+}$ and the mentioned above >99% of identity among all *qepA* alleles; it is need to consider as a limitation the lack of information about the specific efflux activity of the new described Qep variants.

Twelve GenBank sequences were found as presenting recording errors. Thus, in addition to the 4 aforementioned non-identified QepA determinants, 5 QepA amino acid sequences were recorded with an incorrect initial ATG (3 QepA1, 1 QepA4 and a new QepA variant) and 3 QepA4 were identified as QacA.

Although outside the scope of the analysis, the presence of other QepA variants among partial QepA sequences was also observed, as demonstrated by the GenBank sequence CDU26477 which presented 3 amino acid differences regarding QepA1, including E50G which is absent in all fully sequenced QepA variants.

These results show the presence of a high number of unrecognized than recognized QepA variants. Furthermore, they show the presence of additional partially sequenced new variants. While the present proposed allele designation was performed to provide a rational to detected new QepA variants, the establishment of allele assignation rules and the development of a structured repository to bring order to QepA nomenclature seems necessary to avoid future chaos in the scientific literature, especially considering the exponential development of genomic studies.

It is of note that different chromosomal sequences of 512 amino acid residues belonging to soil and water living members of the Comamonadaceae family (such as *Pseudorhodoferax* spp) presenting overall amino acid identity values >80% with QepA1 reaching values of 84% when the comparison was limited to an internal region of 490 amino acid residues. In addition, degrees of identity of around 60-66% with other members of this (e.g.: *Ramlibacter tataouinensis*. *Hydrogenophaga pseudoflava*) or other families (e.g.: *Pigmentiphaga* spp.) belonging to the order Burkholderiales were also observed. Yamane *et al* [2] proposed the ancestral origin of *qepA* in members of the Actinomycetales order considering their similarities in amino acid sequences but, when analyzed, the maximum amino acid identity level between QepA and proteins belonging to these microorganisms was ~40%, reaching 46% when comparison was limited to an internal region of QepA of 493 amino acids. In addition to identity levels, the G+C content (~72%) has also been considered as concordant with a possible origin among Actinomycetales [2]. Nonetheless, the few data available on genus *Pseudorhodoferax* showed similar values of G+C (68-70%) [17,18]. Interestingly, on performing a phylogenetic tree Yamane et al., [2] also observed that a putative efflux pump of *Polaromonas* sp., another member of the Comamonadaceae family, was the most closely related to QepA. Despite ~80% of identity is not enough to define an ancestor, these results suggest that the *qepA* ancestor may be an unidentified member of the Burkholderiales order, probably belonging to the Comamonadaceae family, within or closely related to the *Pseudorhodoferax* genus. Although unfortunately no data about its chromosomal/plasmid location or possible sequence differences were found, the recent detection of QepA in an *Acidovorax* spp., also a member of Comamonadaceae, may be related to this possible ancestral source [13].

In summary, a series of currently undescribed QepA variants have been detected, leading to the identification of 10

Table 1 Characterization of the QepA variants

			GenBank[a]			Amino acid differences													
Variant[b]	N[c]	Year[d]	DNA	Amino acid	I[e]	F95	A99	L102	V134	V185	A235	P274	W318	M372	A445	A507	Deletion	Insertion	Reference[f]
QepA1	21	2007	AB263754	BAF63420	100	F95	A99	L102	V134	V185	A235	P274	W318	M372	A445	A507	Deletion	Insertion	1,2
QepA2	1	2008	EU847537	ACF70723	99.6		G		I										4
QepA3	6	2015	JQ064560	AEZ36150	99.0						E	L	C	K	T				5
QepA4	9	2017	KX580704	AQM56008	99.6	L			I										6,7
QepA5	1	2008	FJ167861	ACI16367	99.8											T			9
QepA6 G[g]	2	2015	NZ_NXMU01000067	WP_096941464	99.8				I										10
QepA7 G	1	2016	NZ_CP014320	WP_064768701	99.8			F											11
QepA8 G	2	2018	CP029249	AWK67508	99.4				I									357LG	-
QepA9 G	1	2018	NZ_CP030329	WP_112021705	99.4	L			I	M									-
QepA10 G	1	2018	NZ_BGSQ01000099	WP_113334341	99.0												432SAALP		-

[a]Both DNA and amino acid sequences belong to the same GenBank record. In all cases the sequences selected are representative of the recorded variant. All QepA variants, except QepA8 which has a 2 amino acid insertion and QepA10 which has a 5 amino acid deletion, present a size of 511 amino acids.

[b]In bold QepA variants reported and numbered in the literature. The allelic variant of unreported or undesignated QepA variants was assigned following the criteria described in the text.

[c]Number of Qnr amino acid sequences present in GenBank.

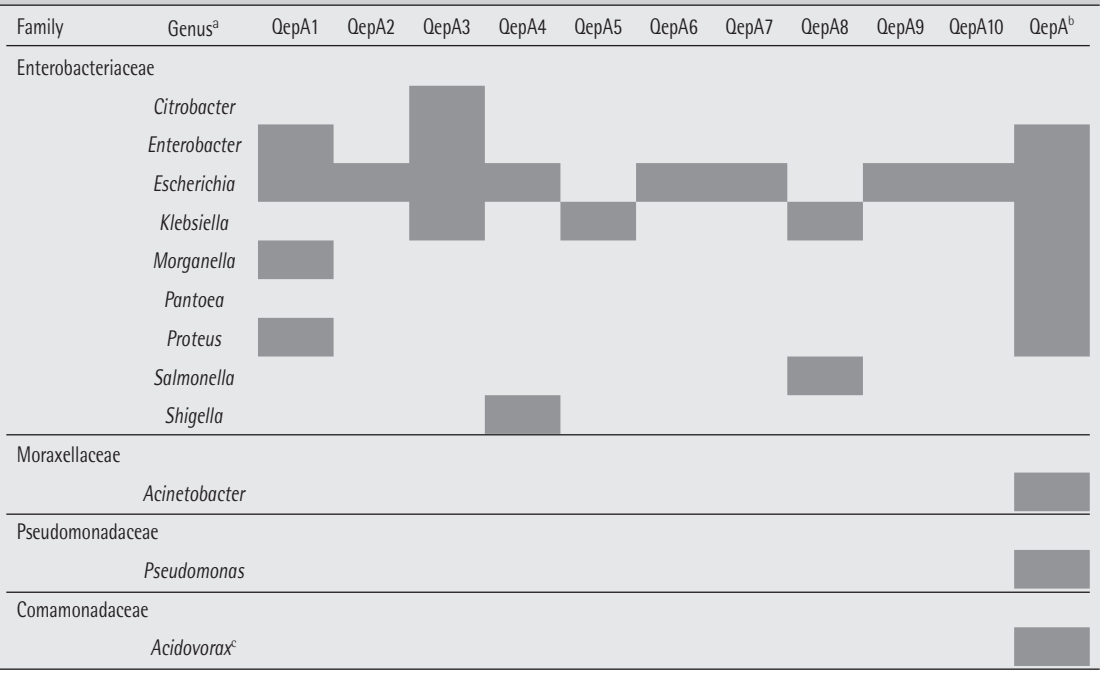
[d]First description. When available, year refers to date of manuscript publication. When no reporting article was found, it refers to date of GenBank submission.

[e]Amino acid identity levels with respect to QepA1.

[f]The symbol "-" denote that no article reporting this sequence has been found.

[g]The "G" notes that these sequences have only been described in genomic studies.

Table 2 **Representative microorganisms in which the presence of transferable QepA has been detected.**



The absence of a specific microorganism in the list does not preclude the non-description of QepA.

[a]**Alphabetical order.**

[b]**Presence of non-specified QepA variants.**

[c]**Although included in the list, if qepA ancestral source is definitively established within Comamonadaceae, the transferable or non-indigenous nature of this qepA gene will be necessary to be established.**

transferable QepA variants and highlighting the need for the establishment of QepA nomenclature rules. Furthermore, the origin of *qepA* among members of the Burkholderiales order can be suspected, suggesting an environmental origin of the *qepA* gene.

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CONFLICT OF INTERESTS

The author declare no conflict of interests.

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