The Class A Carbapenemases BKC-1 and GPC-1 Both Originate from the Bacterial Genus Shinella

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ABSTRACT

Comparative genomics identified the environmental bacterial genus Shinella as the most likely origin of the class A carbapenemases BKC-1 and GPC-1. Available sequences and PCR analyses of additional Shinella species revealed homologous β-lactamases showing up to 85.4% and 93.3% amino acid identity to both enzymes, respectively. The genes conferred resistance to β-lactams once expressed in Escherichia coli. blaBKC-1 likely evolved from a putative ancestral Shinella gene with higher homology through duplication of a gene fragment.

KEYWORDS

class A, β-lactamase, carbapenemase, origin, environment, Gram-negative bacteria, antimicrobial resistance, antibiotic resistance

The high potential of Gram-negative bacteria to acquire exogenous DNA through horizontal gene transfer has allowed clinically relevant bacteria to acquire resistance toward many antibiotics (1, 2). The acquisition of carbapenemase genes in Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii represents one of the most important threats, compromising the use of the entire β-lactam family. Recently, two novel carbapenemase genes, blaBKC-1 and blaGPC-1, were characterized (3, 4). The genes code for weak class A carbapenemases sporadically identified in Klebsiella pneumoniae and P. aeruginosa isolates, respectively. The two enzymes, BKC-1 and GPC-1, share 77% amino acid identity, but their exact origins remain unknown. The aim of this study was to investigate the origin of both blaBKC-1 and blaGPC-1.

All bacterial genomes and plasmids (n = 610,187, downloaded March 2020) available in GenBank were searched for the blaGPC-1 and blaBKC-1-like genes, using DIAMOND v0.9.24.125 at a 70% identity cutoff (5). The blaGPC-1/BKC-1-like genes were identified in 19 assemblies and plasmids. In addition to the presence of blaBKC-1 in the originally reported plasmid from K. pneumoniae, the most similar sequences were found in two Shinella zoogloeoides chromosomes (81.7% and 85.4% amino acid [aa] identity, but if the duplication of part of the gene sequence is considered, the identity is up to 90.2%; see Discussion). The blaGPC-1-like genes were found in 14 different Shinella spp. genomes (S. granuli, S. kummerowiae, S. curvata, Shinella spp.; 80.1 to 93.3% aa identity), and two genomes whose global average nucleotide identity (gANI) analysis showed they are likely to be related to Shinella species and may have been misnamed (Sinorhizobium sp. RAC02 [84.9% aa identity] and uncharacterized Rhizobiaceae bacterium UBA3138 [78.9% aa identity]). Analysis of the two genes’ genetic environments in K. pneumoniae and P. aeruginosa showed the previously demonstrated association with both insertion sequences and plasmid-specific genes (Fig. 1). On the contrary, no insertion sequence or other genes indicating mobility could be associated with the homologues of blaGPC-1/BKC-1 found in Shinella spp. The blaGPC-1 and the blaBKC-1 homologs found in different Shinella spp. were located on the same chromosomal locus as indicated by strong synteny (Fig. 1). Sequence dissimilarities of 7% to 20% across...
this locus between species indicate a long-standing association with the Shinella genus (Fig. 1). Moreover, analysis of the sequences immediately up- and downstream of \( \text{bla}_{ \text{GPC-1} } \) and \( \text{bla}_{ \text{BKC-1} } \) showed high homologies with the corresponding loci in Shinella. The upstream and downstream regions of the \( \text{bla}_{ \text{GPC-1} } \) gene shared 85% (44/52) and 86% (48/56) nucleotide identity with \( S. \text{granuli} \) DD12, while for the \( \text{bla}_{ \text{BKC-1} } \) gene, the corresponding identities to \( S. \text{zoogloeoides} \) were 80% (12/15) and 87% (87/100), respectively.

The GC content of the \( \text{bla}_{ \text{BKC/GPC}} \)-like genes from all Shinella isolates and their mobile counterparts ranged from 65.3% to 69.7%. This overlaps with that of the larger (\( \pm \)10,000 bp) genetic contexts in Shinella but not with that of clinical species carrying \( \text{bla}_{ \text{BKC/GPC}} \) genes (59.3% to 60.5%).

Altogether, this indicates that the two resistance genes share an ancestor gene that have evolved separately into a more \( \text{bla}_{ \text{BKC-1}} \)-like gene in \( \text{S. granuli} \) and a more \( \text{bla}_{ \text{GPC-1}} \)-like gene in, e.g., \( \text{S. granuli} \). It is therefore highly plausible that that \( \text{bla}_{ \text{GPC-1}} \) and \( \text{bla}_{ \text{BKC-1}} \) were mobilized from different Shinella spp.

To evaluate the phenotype provided by the \( \text{bla}_{ \text{BKC/GPC}} \)-like gene and to find other variants and potential closer homologs, four different Shinella species were recovered from the public bacterial collection bank of the Culture Collection of the University of Gothenburg (CCUG), being \( S. \text{granuli} \) 56487, \( S. \text{kummerowiae} \) 56777, \( S. \text{zoogloeoides} \) 35204, and \( S. \text{fusca} \) 55808 (6–8). PCR experiments using degenerate primers were performed, and amplified \( \beta \)-lactamase genes (named \( \text{bla}_{ \text{BKC/GPC}}, \text{bla}_{ \text{GPC}} \), and \( \text{bla}_{ \text{BKC}} \), respectively) were cloned into \( E. \text{coli} \) TOP10 using the pCR2-TOPO cloning kit (Thermo Fisher) and tested for the resistance phenotype using broth microdilution. A CarbaNP test (9) confirmed the ability of the GPC/BKC-like expressing clones to hydrolyze imipenem. All clones displayed a resistance profile against 1-thiactam, including amino- and ureidopenicillins, first- and second-generation cephalosporins and a low level of resistance against third- and fourth-generation cephalosporins, monobactam, and...
TABLE 1 MICs of the clones expressing the different BKC and GPC variants

| Clone or strain | AMX  | AMC  | PIP  | CEF  | FOX  | CXM  | CTX  | CAZ  | CZA  | FEP  | ATM  | IPM  | MEM  | ERT  |
|-----------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| E. coli TOP10   | 4    | 4    | 2    | 4    | 4    | 0.125| 0.25 | 0.25 | <0.125| 0.125| 0.125| <0.125| <0.125| <0.125|
| pGPC-1          | >256 | 6    | 64   | 256  | 32   | 1    | 0.25 | 2    | 1    | 0.25 | 1    | 0.125| 0.125| <0.125|
| pBK-1           | >256 | 4    | 32   | >256 | 32   | 4    | 0.25 | 1    | 2    | 1    | 0.125| 0.125| <0.125| <0.125|
| pBK-b           | 32   | 6    | 4    | 32   | 64   | 4    | 2    | 0.5  | 0.25 | 0.125| 0.5  | 0.25 | <0.125| <0.125|
| pGP-1           | >256 | 6    | 16   | 8    | 32   | 1    | 0.5  | 0.25 | 0.25 | 1    | 0.5  | <0.125| <0.125| <0.125|
| pGPK            | >256 | 8    | >256 | >256 | 32   | 0.5  | 0.25 | 0.75 | 8    | 1    | <0.125| <0.125| <0.125| <0.125|
| pGPZ            | >256 | 4    | >256 | >256 | 32   | 1    | 0.25 | 0.25 | 16   | 2    | 0.25 | 0.25 | 0.25  | 0.25|
| pGPF            | >256 | 6    | 128  | >256 | 128  | 4    | 0.5  | 0.25 | 0.25 | 2    | 1    | <0.125| <0.125| <0.125|

4AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; PIP, piperacillin; CEF, cephalothin; FOX, cefoxitin; CXM, cefuroxime; CTX, cepotaxime; CAZ, ceftazidime; CZA, ceftazidime-avibactam; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; ERT, ertapenem.

carbenamens. All clones remained susceptible to the cephapycin cefoxitin. In addition, the use of clavulanic acid or avibactam restored a complete susceptibility against amoxicillin or ceftazidime, respectively, a characteristic shared by class A β-lactamases (Table 1). This phenotype is in accordance with those reported for BKC-1 and GPC-1 (3, 4). Protein alignments using SeaView Software (Prabi, Doua, France) showed that GPC-1 was most closely related to the GPC-like proteins from Shinella sp. strain DD12 (93.3%) and S. granuli (92.6%), whereas BKC-1 shared the highest amino acid identity with S. zoogloeoides (85.4%) (see Fig. S1 in the supplemental material). All variants possessed the typical conserved serine/threonine kinase motifs and the motif involved in the Ω-loop formation of class A β-lactamases (10) (Fig. 2). Deeper alignment analysis showed that BKC-1 displayed a duplication of 16 amino acids, being the repetition of the protein segment from Ala12 to Ser27. Therefore, a putative ancestral protein was designed in silico and named BKC-b (Fig. 2). Aligning the BKC-b sequence with GPZ increased the amino acid identity up to 90.2% compared to 85.4% without the duplication (Fig. S1). Production of BKC-b in E. coli TOP10 showed a weaker resistance profile while having an increased activity against cefoxitin. The use of the I-TASSER (11) in silico tool predicted the tridimensional structures of both BKC-b and BKC-1 and showed that the duplication of the protein segment in BKC-1 modified the ligand binding site of the enzyme and probably led to the increased spectrum of activity observed in BKC-1 compared to that in BKC-b but the loss of its activity against cephamycins.

Here, we provide evidence that the genes blaGPC-1 and blaBKC-1 were most likely mobilized from members of bacterial genus Shinella into clinical species. This conclusion is based on the presence of a conserved locus containing a blaGPC/BKC-like gene in all investigated Shinella species, the lack of associated mobile genetic elements, and high amino acid and nucleotide identities to the clinical counterparts, but not so high that we could assign with confidence the exact origin species. However, it is highly plausible that the origins of blaGPC-1 and blaBKC-1 are Shinella species closely related to S. granuli and S. zoogloeoides, respectively. The resistance phenotype provided by the blaGPC/BKC-like genes is in line with mobilization and transfer driven by antibiotic exposure. The Shinella genus includes mesophilic, aerobic Gram-negative species mainly recovered from environmental samples. For instance, the studied S. granuli and S. zoogloeoides isolates were recovered from sludge in China, while the S. kummerowiae and S. fusca isolates were recovered from root nodules and domestic compost in Korea and Portugal, respectively (6–8). The presence of a natural and functional β-lactamase gene in this genus could be explained by the presence of β-lactam-producing microorganisms sharing the same niche (12). Additionally, we show that the BKC-1 protein presented a duplication of its Ala12-Ser27 segment, likely from a putative ancestral protein BKC-b. Hence, the blaBKC-1 gene may have evolved from blaBKC-b likely under a selective pressure from β-lactams, eventually resulting in a more efficient enzyme. This mutation led, on the other hand, to the reduction of its activity against cefoxitin.
Emergence of new resistance genes, especially genes providing resistance to antibiotics of last resort, such as carbapenems, represents a major clinical threat. After initial emergence, they are likely to remain undetected and spread silently in the human microbiota for some time. When detected, they are often already widespread (13).

Understanding the origin and mobilization history of as many and diverse clinically important resistance genes as possible could enable us to manage risks for future emergence events in a better way. The data presented here provides one additional piece in this large puzzle.

**Data availability.** The nucleotide sequence of the carbapenemases genes \(\textit{bla}_{\text{GPG}}\), \(\textit{bla}_{\text{GPZ}}\), \(\textit{bla}_{\text{GPF}}\), and \(\textit{bla}_{\text{KCC}}\) were submitted to GenBank with the following accession numbers, respectively: MT661611, MT661612, MT661613, MT661614, and MT661610.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.**

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