Emergence of *Klebsiella pneumoniae* Coproducing KPC-2 and 16S rRNA Methylase ArmA in Poland

Katarzyna Zacharczuk,1 Katarzyna Piekarska,1 Jolanta Szych,1 Elwira Zawidzka,2 Agnieszka Sulikowska,2 Sebastian Wardak,2 Marek Jagielski,1 and Rafał Gierczynski1*

Department of Bacteriology, National Institute of Public Health–National Institute of Hygiene, Chocimska 24, 00-791 Warsaw, Poland; and Niepubliczny Zakład Opieki Zdrowotnej, Laboratorium Analiz Lekarskich–ALAB, Sokolowska 7, 01-142 Warsaw, Poland.

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A *Klebsiella pneumoniae* epidemic strain that coproduced carbapenemase KPC-2 (*K. pneumoniae* carbapenemase 2) and 16S rRNA methylase ArmA has emerged in Poland. Four nonduplicate isolates from patients in a hospital in Warsaw, Poland, were found to carry the *bla*<sub>KPC-2</sub> and *armA* genes on ca. 50-kb and 90-kb plasmids, respectively. Tn<sub>4401</sub> with a 100-bp deletion in the variable region was detected in all the isolates. XbaI pulsed-field gel electrophoresis (PFGE) revealed 93.2% similarity of the isolates. All the isolates were resistant to carbapenems and 4,6-disubstituted 2-deoxystreptamines.

*Klebsiella pneumoniae* carbapenemase (KPC) is a molecular class A serine β-lactamase belonging to functional group 2f. This enzyme hydrolyzes β-lactams of several different classes, including carbapenems (18). KPC was first reported in the United States in 2001 (18, 25). This enzyme is encoded by the *bla*<sub>KPC</sub>-gene, which maps to transposon Tn<sub>4401</sub> that has been reported to occur in a variety of transferable plasmids (12, 16). Despite a highly conserved amino acid sequence of KPC, several variants of the *bla*<sub>KPC</sub>-gene have been distinguished (http://www.lahey.org/Studies/other.asp#table1). KPC has recently become a major therapeutic challenge with the KPC-2 and KPC-3 variants predominating in the United States and Europe (14, 18, 19, 23). Besides clonal dissemination in hospitals (17), *bla*<sub>KPC</sub> has also been found to spread laterally, even at the interspecies level (20). *Enterobacter cloacae* producing KPC-2 together with 16S rRNA methylase ArmA was recently reported in China (23).

Plasmid-encoded 16S rRNA methylases have emerged in members of the family *Enterobacteriaceae* as a new mechanism of resistance to 4,6-disubstituted 2-deoxystreptamines, which encompass a majority of clinically important aminoglycosides (24). In contrast to substrate-specific aminoglycoside-modifying enzymes, the methylases confer high-level resistance by altering the 16S rRNA A site. Among 16S rRNA methylases described thus far (5), ArmA is a global concern (7, 9, 15). This methylase was first detected in *Citrobacter freundii* in Poland (11) and currently predominates in Europe (2, 11). ArmA is encoded by the *armA* gene (8), which maps to composite transposon Tn<sub>5489</sub> (9) located on a large (ca. 80- to 90-kb) conjugative plasmid that also carries a gene encoding a CTX-M-type extended-spectrum β-lactamase (ESBL) (11).

Herein, we report emergence of *Klebsiella pneumoniae* co-producing carbapenemase KPC-2 and 16S rRNA methylase ArmA in Poland. To the best of our knowledge, this is the first such report in Europe.

We tested five nonduplicate clinical isolates of *K. pneumoniae* collected from patients in a hospital in Warsaw, Poland. All five isolates were considered etiologic agents of infections, mostly urinary tract infections (see Fig. 1A for details). The isolates were identified using Vitek 2 (bioMerieux, France) and were found resistant to ertapenem and meropenem. Four isolates were also resistant to various aminoglycosides. Agar dilution suggested 16S rRNA methylase activity. Consequently, all the tested isolates were examined by PCR (8, 12) for the *bla*<sub>KPC</sub> and *armA* genes. The *bla*<sub>KPC</sub>-gene was detected in all isolates tested, while *armA* was found in four isolates (Table 1). The *bla*<sub>KPC</sub>-variant expressed by the isolates tested was determined by DNA sequencing with primers KPC895F (5'-TTGTAGTGTCACTGTATCGCCG-3') and KPC895R (5'-TTCAGAGCCTACTGCCCCG-3') (the forward and reverse primers are indicated by F and R at the end of the primer designation, respectively) using the BigDye terminator V.3.1. cycle sequencing kit (Applied Biosystems, Foster City, CA). The primers were designed for the reference *bla*<sub>KPC-2</sub>-gene sequence (GenBank accession number AY034847). All strains tested were found to carry the *bla*<sub>KPC-2</sub>-gene for KPC-2. This variant of KPC has been recently reported in Poland in *K. pneumoniae* ST258 (1). Therefore, we determined the multilocus sequence type of isolate DM0269 according to the protocol described on the *K. pneumoniae* multilocus sequence typing (MLST) website (http://www.pasteur.fr/recherche/genopole/PGF/MLST/Kpneumoniae.html). Our results showed that isolate DM0269 also belongs to the ST258 clone.

Plasmids were extracted from the tested strains using the NucleoBond PC 20 kit (Macherey-Nagel, Germany). Four tested strains harbored three large plasmids (Fig. 1B) with...
estimated sizes of 50, 90, and 180 kb. Isolate DM0267, which was armA negative, lacked the 90-kb plasmid. Southern blot analysis was performed as previously described (22) to determine which plasmids carry the \textit{bla} \textit{KPC-2} and \textit{armA} genes. The probes specific for these two genes hybridized with 50-kb and 90-kb plasmids, respectively (Fig. 1B). Multiple electroporation experiments were carried out to transform \textit{Escherichia coli} DH5\textalpha transformants. ET\textit{bla} \textit{KPC} and ET\textit{armA}, plasmids pETKp50 and pETKp90 from isolate DM0269, respectively. Lane W, plasmids from the wild donor. (D) PstI restriction endonuclease profiles of plasmids in \textit{E. coli} DH5\textalpha transformants with bands to which \textit{bla} \textit{KPC} and \textit{armA} probes hybridized. PstI profiles of plasmids from ET\textit{bla} \textit{KPC} (lane 1), ET\textit{armA} (lane 3), and \textit{E. coli} \textit{Δ32/01} (lane 5) together with Southern blot hybridization with \textit{bla} \textit{KPC} (lane 2) and \textit{armA} (lanes 4 and 6) probes. Lane M, DNA ladder (λ phage HindIII digested). The approximate size of DNA is shown in kilobases to the left of the gels in panels B to D. The picture was electronically edited to show DNA electrophoretic patterns and the corresponding Southern blot hybridization results together.

Other studies have shown that the \textit{bla} \textit{KPC-2} gene occurs in a variety of plasmids ranging from 24 to 120 kb (1, 12). Nevertheless, it was most frequently reported in plasmids from 50 kb to 75 kb (14, 18), which is in concordance with our results. In contrast, human clinical isolates producing ArmA were reported to bear the \textit{armA} gene on a conjugative plasmid of ca. 90 kb that was similar to \textit{pCTX-M3} (11). Because \textit{pCTX-M3} carries a gene encoding \textit{CTX-M-3} ESBL, we carried out PCR assay with primers specific for the \textit{bla} \textit{CTX-M} gene family (6). All the isolates tested except for DM0267 were found to produce \textit{CTX-M-type} ESBL (Table 1). Since \textit{pCTX-M3} and its derivatives were commonly detected in Enterobacteriaceae in Poland, it may explain the presence of the \textit{pCTX-M3-type} plasmids in the isolates tested (2, 9, 11). In contrast, the origin of the plasmid carrying the gene encoding KPC-2 remains unclear. The only KPC-producing \textit{K. pneumoniae} isolates reported so far in Poland carried the \textit{bla} \textit{KPC-2} gene on a large plasmid that was 110 kb in size (1). The isolates tested, however, bear this gene on the 50-kb plasmid. To better characterize this plasmid, PCR assay with primers specific for non-conserved region of the Tn\textit{4401} was carried out (14). All the

![FIG. 1. (A) Dendrogram of the tested \textit{K. pneumoniae} isolates based on XbaI PFGE profiles. The XbaI PFGE pattern, isolate, presence (+) or absence (−) of the \textit{armA} gene, ward, and date (sample collection date) are shown. URL, urological department; INT, internal diseases department; Tol, tolerance. (B) Plasmid profiles of \textit{K. pneumoniae} isolates with \textit{bla} \textit{KPC} and \textit{armA} carriers delineated by the Southern blot DNA/DNA hybridization. Lanes 1 to 5 show the plasmid profiles of strains DM0267, DM0270, DM0265, DM0266, and DM0269, respectively. Lanes 6 and 7 show the hybridization results for the plasmid profile of isolate DM0269 and molecular probes specific for \textit{bla} \textit{KPC} and \textit{armA}, respectively. Chr, chromosomal DNA fragments. (C) Plasmids in \textit{E. coli} DH5\textalpha transformants. ET\textit{bla} \textit{KPC} and ET\textit{armA}, plasmids pETKp50 and pETKp90 from isolate DM0269, respectively. Lane W, plasmids from the wild donor. (D) PstI restriction endonuclease profiles of plasmids in \textit{E. coli} DH5\textalpha transformants with bands to which \textit{bla} \textit{KPC} and \textit{armA} probes hybridized. PstI profiles of plasmids from ET\textit{bla} \textit{KPC} (lane 1), ET\textit{armA} (lane 3), and \textit{E. coli} \textit{Δ32/01} (lane 5) together with Southern blot hybridization with \textit{bla} \textit{KPC} (lane 2) and \textit{armA} (lanes 4 and 6) probes. Lane M, DNA ladder (λ phage HindIII digested). The approximate size of DNA is shown in kilobases to the left of the gels in panels B to D. The picture was electronically edited to show DNA electrophoretic patterns and the corresponding Southern blot hybridization results together.](http://aac.asm.org/)

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isolates tested yielded a ca. 604-hp amplicon corresponding to a Tn4401 variant with a 100-bp deletion (data not shown). Interestingly, this variant is relatively rare. It was found in K. pneumoniae ST258 in the United States and Israel (14). The results of the transposon analysis suggested that the isolates tested were clonal. Pulsed-field gel electrophoresis (PFGE) was therefore conducted (14). PFGE patterns were analyzed using GelCompar II version 5.10 software (Applied Maths, Sint-Martens-Latem, Belgium). Four genotypes were distinguished (Fig. 1). The very high similarity (93.5%) of the genotypes suggested that all the isolates tested belong to the same strain sensu stricto.

In conclusion, our results demonstrate that K. pneumoniae in Poland has accumulated two broad-spectrum resistance traits: KPC carbapenemase and 16S rRNA methylase ArmA. Due to its resistance to ciprofloxacin, cephalexins, carbapenems, and a spectrum of aminoglycosides, this strain disseminated among patients in two hospital wards and became a real challenge for the hospital authorities. Our findings suggest that coproduction of KPC-2 and ArmA is a novel strategy developed by K. pneumoniae to survive in hospitals if aminoglycosides and carbapenems are administered in combination. This hypothesis is also supported by Jiang and coworkers (13), who found a clinical isolate of K. pneumoniae that carried blaKPC-2 and armA in a large multidrug resistance plasmid in China. Moreover, strong selective pressure favoring coproduction of KPC-2 and ArmA by hospital strains was also observed in a clinical isolate of Enterobacter cloacae in China (23). This isolate carried blaKPC-2 and armA on two large plasmids, which resembles the phenomena reported herein for K. pneumoniae.

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REFERENCES


