The emergence of *Klebsiella pneumoniae* ST258 with KPC-2 in Poland

Plasmidic β-lactamases KPC in Gram-negative pathogens are of the highest clinical and epidemiologic concern, conferring resistance to all β-lactams, including carbapenems [15, 18]. The main KPC producer is *Klebsiella pneumoniae*, but other species are being identified as well [15]. Strains with KPCs spread rapidly and cause outbreaks; in hospitals on the east coast of the USA and in Israel they have already become endemic [2, 3, 10, 14, 17]. Recently, more countries reported these organisms, in some cases as a result of importation [15].

On the 19th of May, 2008, a 56-year-old patient with no travel history was admitted to a cardiology ward in a Warsaw hospital (H1) with ischemic cardiomyopathy and several other co-morbidities. He had been transferred from another hospital with pneumonia of unknown etiology, treated empirically with ceftriaxone and ciprofloxacin until the 28th of May. The eradication of pneumonia was asserted by clinical criteria. However, on the 23rd of May, the patient developed a urinary tract infection (UTI) without a bladder catheter caused by *K. pneumoniae* (isolate 2337/08). With imipenem MIC of 2 µg/ml the isolate was classified by Vitek 2 Expert (bioMérieux, Marcy l’Etoile, France) as resistant and of the ‘ESBL + carbapenemase’ phenotype. The next such isolate (2338/08) was recovered from urine collected on the 28th of May, two days after the start of treatment with amikacin. An environmental sampling, conducted on the same day in the patient’s room, yielded a similar *K. pneumoniae* from a basin (isolate 2641/08). On the 3rd of June, the eradication of the UTI with amikacin was proven through clinical criteria and microbiological examination, and the patient was discharged, without having been tested for fecal carriage. On the 6th of June, he was admitted to a cardiology center (H2) for heart transplantation. At the time of admission his urine, stool and sputum were analyzed. *K. pneumoniae* was only cultured from the stool (isolate 2639/08). The patient was kept under strict isolation without antimicrobial treatment, and his stool was examined each day during the first week, then twice a week during the
remaining hospitalization. The stool samples were analyzed by standard procedures for enterics, followed by speciation with the ATB ID32E test (bioMérieux) and susceptibility testing by disk-diffusion according to the CLSI [6], including imipenem, meropenem, and ertapenem. On the 27th of June, the sample was finally free of *K. pneumoniae*, and this was confirmed in several subsequent analyses. On the 17th of July, the patient, disqualified for heart transplantation, was discharged from center H2 without symptoms of infection.

Analyzed by Etest (AB Biodisk, Solna, Sweden), the isolates showed multi-resistance, including reduced susceptibility to carbapenems (Table 1). In the spectrophotometric assay [17] bacterial sonicates hydrolyzed imipenem in the EDTA-independent manner. Isoelectric focusing [1] visualized β-lactamases with pIs of 7.6 (probably the SHV-like chromosomal enzymes) and three others, with pIs of 8.2, ~6.8 and 5.4. Since the pI ~6.8 specifies the KPC-2/-3 β-lactamases, a PCR [13] and sequencing were performed, which identified the *bla*KPC-2 genes [19]. PCR mapping revealed that they resided in one of the Tn4401 transposon variants, Tn4401a [12]. Mating was performed with *Escherichia coli* A15 RifR [1]; transconjugants were selected with 100µg/ml rifampin and 0.5µg/ml imipenem or 2µg/ml ceftazidime. Only isolate 2641/08 gave a transconjugant on the imipenem plate with KPC-2 and the pI 5.4 enzyme, identified by PCR and sequencing [9] as TEM-1. Contrarily, all isolates yielded transconjugants on ceftazidime plates which had only the 8.2 enzymes, found by PCR and sequencing [9] to be extended-spectrum β-lactamase (ESBL) SHV-12. Plasmid DNA of the isolates, purified with the QIAGEN Plasmid Midi Kit (QIAGEN, Hilden, Germany), was used for electroporation of *E. coli* DH5α, analyzed then on 0.5µg/ml imipenem plates. Transformants with KPC-2 and TEM-1 were obtained for all isolates. PstI fingerprinting [9] showed three plasmid molecules in each isolate, two of which segregated to SHV-12-producing transconjugants and KPC-2/TEM-1-producing transformants, respectively (Figure 1). Sizes of the plasmids were evaluated by calibration of the fingerprint bands and by pulsed-
field gel electrophoresis (PFGE) of total DNA digested with nuclease S1 [16]. Plasmids with
the \textit{bla}_{SHV-12} gene and with the \textit{bla}_{KPC-2} and \textit{bla}_{TEM-1} genes had \textasciitilde40kb and \textasciitilde110kb,
respectively, whereas those likely without \textit{bla} genes were of \textasciitilde200kb. The PCR-based replicon
typing (PBRT) [5] yielded products only with primers specific for the replicon FIIA, of
\textit{Salmonella} virulence plasmids [5]. Moreover, it worked only with DNA of the \textit{K. pneumoniae}
isolates and not of the transconjungants and transformants, indicating that the amplicons
corresponded to the plasmids likely without \textit{bla} genes. Sequencing of the amplicons revealed
their ca. 97\% identity with fragments of \textit{repA} genes of plasmids pKPN3 and pKPN4 from \textit{K. pneumoniae}
MGH78578 (GenBank CP000648 and CP000649), and only ca. 79\% with plasmid pSLT from \textit{S. Typhimurium} LT2 [11]. \textit{K. pneumoniae} isolates were indistinguishable
by PFGE of their DNA cut with the XbaI restriction enzyme [9]. Multilocus sequence typing
(MLST) [8, \texttt{www.pasteur.fr}] classified them into sequence type ST258, recently identified for
KPC producers in Norway and Sweden, mostly of the Greek and Israeli origins [16].
This first report on KPC producers in Poland documents their continuing spread. The strain
belonged to a wide-spread clonal group with single-locus variants ST11, ST258, and ST270
[7, \texttt{www.pasteur.fr}]. The previous ribotyping- and PFGE-based observations on high clonality
of KPC-producing \textit{K. pneumoniae} [4, 14, 17], and the MLST data from this and the
Scandinavian work [16] suggest a prominent role of this group in KPC dissemination.
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<th>Isolate</th>
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<tr>
<td></td>
<td>AMP</td>
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<tr>
<td>clinical isolates</td>
<td>&gt;256</td>
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<tr>
<td>transcon. 2641/08</td>
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<td>&gt;256</td>
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<td>E. coli A15</td>
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*Abbreviations: AMK, amikacin; AMC; amoxycillin-clavulanate; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; CTX, cefotaxime; FEP, cefepime; GEN, gentamicin; IPM, imipenem; MEM, meropenem; PIP, piperacillin; TET, tetracycline; TGC, tigecycline; TZP, piperacillin-tazobactam.*

*In the case of β-lactam antibiotics, the MICs shown are the values indicated by zones of the confluent growth around Etest strips; the clinical isolates and the E. coli transformants with KPC-2 produced scattered colonies inside the growth inhibition zones [15].*
FIGURE LEGEND

FIGURE 1.

The PstI (Fermentas, Vilnius, Lithuania) fingerprinting of plasmid DNA from the *K. pneumoniae* clinical isolates, *E. coli* DH5α transformants producing KPC-2 and TEM-1 (T), and *E. coli* A15 transconjugants producing SHV-12 (R). Molecular weight markers: M1, λ DNA/HindIII, and M2, GeneRuler 1kb DNA Ladder (Fermentas). DNA sizes on the left side correspond to the GeneRuler 1kb DNA Ladder, whereas those on the right side – to λ DNA/HindIII.