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First outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* in Germany

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Abstract We report the first outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* in Germany. The presence of KPC was confirmed by polymerase chain reaction (PCR). The KPC-encoding plasmid was analysed by transconjugation experiments, DNA sequencing, Southern blotting and isoelectric focusing. Typing was performed by pulsed-field gel electrophoresis (PFGE). An ertapenem-resistant *K. pneumoniae* with low minimum inhibitory concentrations (MIC) to other carbapenems (tested by the Vitek system) was isolated from the index patient in January 2008. A KPC-2 was identified after *K. pneumoniae* with identical susceptibility patterns had been isolated from two more patients. Despite the introduction of infection control measures, transmission occurred in five additional patients and three of the patients died from infections. The source of the outbreak strain remained

unclear; however, the Tn4401-containing *bla*_{KPC-2} gene was similar to previously described isolates from Greece. Five months after the end of the outbreak, a KPC-*K. pneumoniae* was isolated from a patient who had been treated in Greece previously. Retrospectively, this patient was treated in November 2007 on the same unit as the index case. Typing revealed that all patients were colonised by the same strain. KPC-*K. pneumoniae* has been introduced to Germany possibly from Greece and transmission to other institutions is likely.

Introduction

Carbapenemases of the *Klebsiella pneumoniae* carbapenemases (KPC) type belong to the molecular class A β -lactamases [1]. The first clinical isolate of this enzyme that can hydrolyse carbapenems in addition to all cephalosporins was detected in 1996 in North Carolina [2]. Since then, KPC-producing *K. pneumoniae* has spread with alarming speed, especially in New York and the NY area [3–6]. After the expansion of KPC-*K. pneumoniae* along the east coast of the United States, the detection of KPC was reported from other countries beginning in 2005 [7]. Isolates were reported from South America [8, 9], China [10] and Israel [11–13]. In Europe, individual cases of colonisation or infection with KPC-producing *K. pneumoniae* were reported from France [7], Greece [14, 15], Sweden [16] and the United Kingdom [17]. Recently, an outbreak of KPC-producing *K. pneumoniae* was reported from Greece [18].

The genetic information for KPC is located on a conjugative plasmid, and, apart from *K. pneumoniae*, KPC was additionally detected in *K. oxytoca* [19], *Citrobacter freundii* [20], *Serratia marcescens* [21],

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Escherichia coli [22], *Enterobacter* spp. [23], *Salmonella* spp. [24] and *Pseudomonas aeruginosa* [25]. Recently, the transposon Tn4401 has been proposed to be at the origin of KPC mobilisation [26]. Sequence analyses of isolates from America and Europe revealed, besides a large consensus, few variations in the left and right target site duplication and, in two out of five investigated strains, a 100-bp deletion upstream of the KPC-2 coding sequence.

We report the first outbreak of KPC-producing *K. pneumoniae* in Germany, which affected eight patients, of whom three died from infection. This report describes the outbreak, the infection control measures implemented and the results of molecular investigations to elucidate the origin of KPC in our patients.

Methods

Setting

The University Hospitals of Heidelberg are a 1,600 bed tertiary care centre serving about 52,000 patients per year. All affected patients were nursed in the department of general, visceral and transplantation surgery. The 14-bed interdisciplinary surgical intensive care unit (ICU) is operated in cooperation by the department of anaesthesiology and the department of general, visceral and transplantation surgery. A 14-bed intermediate care unit treats intensive care patients who no longer need mechanical ventilation and patients after transplantation of visceral organs, i.e. liver or renal transplant patients. Patients of these units are routinely screened for methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant enterococci (VRE), but screening for Gram-negative microorganisms is not performed. The infection control team was alerted after three patients were detected who had *K. pneumoniae* with an unusual resistance pattern isolated from tracheal secretion.

Microbiological methods

Enterobacteriaceae isolated from clinical specimens are routinely tested for antibiotic susceptibility by the Vitek 2 system (bioMérieux, Nürtingen, Germany). Susceptibilities were interpreted using the European Committee for Antimicrobial Susceptibility Testing (EUCAST)/BSAC (v.7) breakpoints. The modified Hodge test was performed as described by Lee et al. [27].

Screening specimens for KPC-producing *K. pneumoniae* were cultured on Columbia blood agar, McConkey agar and chromID extended spectrum β -lactamases (ESBLs, bioMérieux). Representative colonies of all suspect enterobacteriaceae were tested by the Vitek 2 system. If the

minimum inhibitory concentration (MIC) to ertapenem was elevated, further testing by polymerase chain reaction (PCR) and the modified Hodge test was performed.

Characterisation of *bla*_{KPC} and Tn4401-like elements

The *bla*_{KPC} gene was detected using PCR primers encompassing the entire coding region, resulting in an amplicon of 893 bp [5]. Template DNA for cycling PCR was obtained by using standard DNA isolation procedures (High Pure PCR Template Preparation Kit, Roche). Both strands of the PCR products were then sequenced (BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems) using the amplification primers and detected on an ABI 310 (Applied Biosystems). The sequencing files were assembled from the resultant chromatograms with the Staden suite (version 1.7.0) software package (Staden, R. 1996. Mol. Biotechnol. 5:233) The sequence data were analysed for similar GenBank entries by the BLAST algorithm [28].

Precise determination of both Tn4401 insertion sites and the variable deletion between the *istB* and the downstream *bla*_{KPC-2} gene, occurring in Tn4401 elements of different geographical origin, was performed by using the primers as described [26].

Plasmids were obtained by a standard alkaline lysis method and analysed on a 1% agarose gel. *E. coli* strain J53 containing the plasmid pMG252 (kindly provided by Dr. G. Jacoby, Lahey Clinic, Burlington, MA 01805) served as the size standard.

Genotyping of isolates

Molecular typing was performed after the digestion of chromosomal DNA with the restriction enzyme *speI* by pulsed-field gel electrophoresis (PFGE) using the contour-clamped homogenous electric field (CHEF) electrophoresis technique and amplified fragment length polymorphism (AFLP) [29]. Clonal diversity was defined as proposed by Tenover et al. [30] and evaluated by Jonas et al. [31].

Conjugation experiments

The PCR-detectable *bla*_{KPC} gene locus of *K. pneumoniae* could be mobilised together with the antimicrobial resistant phenotype into an *E. coli* J53Az^R recipient by trans-conjugation and subsequent selection on 100 μ g/ml Nazide in combination with 100 μ g/ml cefotaxime after overnight filter-mating, as described previously [32]. Antimicrobial susceptibility testing of the donor, recipient and transconjugants was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) [33].

Isoelectric focussing

The β -lactamases produced by the strains were isolated by ultrasonic treatment [34] and characterised with regard to the isoelectric point by isoelectric focussing on a polyacrylamide gel (ampholytes [pH 3–10]) and inhibition by clavulanic acid [35].

Outbreak investigation

All patients who presented KPC-producing *K. pneumoniae* isolated from any body site were defined as cases. Patients who had been nursed in the same room as a case patient were defined as contact patients. If a contact patient was nursed with a case patient on more than one unit, each contact was counted as a single incident. The location and duration of contacts between patients was identified from the hospitals' electronic information system.

The intervention consisted of isolating the affected patients in single rooms and strict barrier nursing was implemented. Health care workers were informed about KPC by information sheets and short informative meetings.

Prevalence screenings were performed on two different occasions. The first screening included all patients who were nursed in the affected units and the second included all patients who were nursed in the affected department during the preceding 10 days.

If case patients were transferred to other institutions, the health care providers of these institutions were informed about the colonisation status of the patients. Furthermore, laboratory and infection control staff of German health care institutions were informed about the identification of KPC in Germany [36].

Results

In January 2008, a *K. pneumoniae* was isolated from a wound specimen of a patient nursed on the interdisciplinary ICU, which was resistant to all β -lactams, fluoro-quinolones, cotrimoxazole, aminoglycosides and ertapenem, but had susceptible MICs to imipenem and meropenem, as measured by the Vitek 2 system. Due to low the MICs to imipenem and meropenem, a carbapenemase was not suspected at this time. A KPC-2-encoding gene locus was identified after two more patients from the same unit had *K. pneumoniae* with identical susceptibility patterns isolated from respiratory specimens. Strict isolation measures were implemented and health care workers were informed about KPC-mediated resistance by leaflets and informative meetings.

Despite the introduction of these measures, during the following months, five additional patients were identified, who were colonised or infected with KPC-producing *K.*

pneumoniae. Therefore, we performed prevalence screenings of potential contact patients on two different occasions including 31 and 51 patients, respectively.

However, all screened patients were tested negative. The last patient associated with the outbreak left the hospital in June and no additional cases were detected during the following six months. All case patients were kept in strict isolation until they left the hospital.

The index patient had no history of travel or contacts to persons from endemic areas. Therefore, it was assumed that the KPC-positive strain may have been introduced by another patient who remained undetected. Case patients had been treated in the hospital for up to 262 days before colonisation or infection with KPC-producing *K. pneumoniae* became apparent. During this time, the case patients were transferred several times between different units (2 to 13 times, median 8.5 times). They had 624 contacts to 481 patients before they were treated in isolation.

Noteworthy, among the 17 patients who had more than three contacts to case patients, seven became case patients later. The duration of contacts was between 1 and 960 h, whereby patients who became case patients had longer contact times with other case patients (mean 109.3 h, range

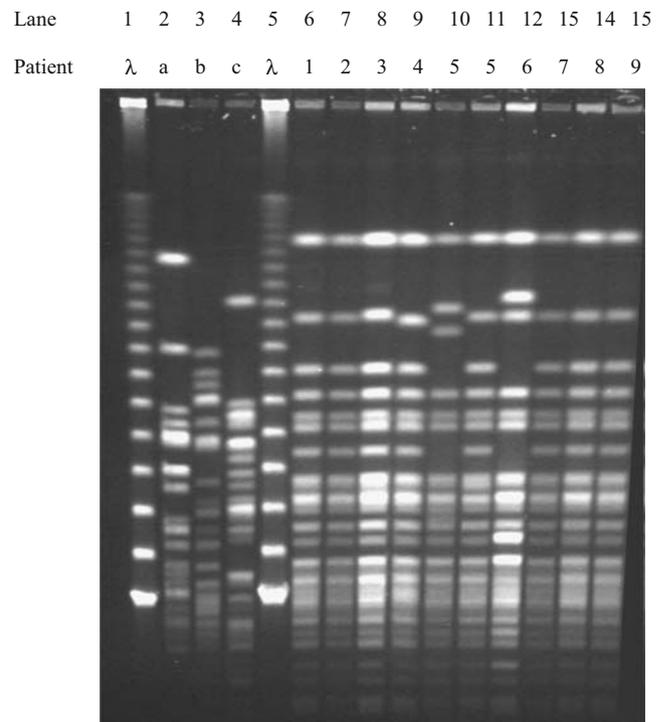


Fig. 1 Pulsed-field gel electrophoresis (PFGE) of *speI*-digested DNA of *Klebsiella pneumoniae*. Lanes 2–4: unrelated non-*K. pneumoniae* carbapenemases (KPC)-producing isolates from three patients a, b, c; lanes 6–15: KPC-producing isolates from case patients 1 to 9; lanes 1 and 5: size markers are concatemers of phage λ DNA (multimers of 48.5 kb)

4–480 h) than other patients (mean 52.3 h, range 1–960 h). This difference was statistically significant ($p = 0.025$).

Almost all of the patients who had been in contact with case patients left the hospital with unknown colonisation status. Thus, unrecognised transfer of the KPC-producing *K. pneumoniae* to other institutions is possible.

Five months after the last case patient had left the hospital, another KPC-producing *K. pneumoniae* was isolated, this time from an abscess in a carcinoma patient who had been treated in Greece previously (case 9). Typing revealed that this patient was infected with an isolate that was indistinguishable from the outbreak isolates (Fig. 1). Retrospectively, this patient was treated for an abdominal abscess in November 2007—shortly before the outbreak—on the same unit as the index case. Unfortunately, no microbiological samples were taken during this in-patient stay.

All case patients suffered from severe underlying diseases: two of them had solid organ transplantation, four suffered from malignancies, two had complication after surgery and one patient had multiple trauma (Table 1).

KPC-producing *K. pneumoniae* caused infections in three patients (pneumonia, wound infection and sepsis). All of these patients were treated with combinations of antibiotics that included colistin. Unfortunately, none of these patients could be successfully treated. An additional case patient died from her underlying disease.

PFGE and AFLP typing concordantly revealed that all patients were colonised by the same *K. pneumoniae* strain, with one patient harbouring a subtype of the outbreak clone and another patient (patient 5) harbouring the outbreak clone and, additionally, a subtype that displayed a different appearance on the agar plates (Fig. 1). MICs of the *K. pneumoniae* isolates to several antibiotics and an additional *E. coli* isolate from the index patient that was also shown to harbour the *bla*_{KPC} gene are shown in Table 2.

Sequencing of the *bla*_{KPC} PCR product allowed to identify a KPC-2 carbapenemase. The isoelectric point of the enzyme was 6.8.

Plasmid preparation from both species revealed cccDNA larger than the plasmid pMG 252 (Fig. 2), which served as an approximate standard with an estimated size of 180 kb

Table 1 Characteristics of the case patients

Patient	Age (years)	Underlying disease	Length of stay (days)		Detection of KPC-producing <i>K. pneumoniae</i>		Infection due to KPC-KP	Therapy	Outcome
			Before detection of KPC-KP	Total	Initial detection	Other culture-positive sites			
1: female	55	Urethral stenosis after radiation therapy	55	78	Wound	Tracheal secretion	Pneumonia	Tigecycline, colistin	Deceased
2: male	42	Multiple trauma	11	29	Tracheal secretion	Throat	–	–	Transferred
3: female	71	Kidney transplantation, colon perforation	86	119	Tracheal secretion	Wound, CVC, urine, throat, rectal	Wound infection, pneumonia	Tigecycline, colistin	Deceased
4: female	68	Arterial obstructive disease, above-knee amputation	53	129	Rectal	–	–	–	Transferred
5: female	55	Liver transplantation	66	157	Rectal	Urine	–	–	Transferred
6: male	72	Oesophageal cancer	147	140	Urine	–	–	–	Transferred
7: female	67	Recurrent ovarian carcinoma	28	33	Wound	–	–	–	Transferred, deceased
8: female	52	Obesity, gastric sleeve operation	262	316	Urine	Tracheal secretion, BAL, throat, wound, rectal	Wound infection, pneumonia, sepsis	Tigecycline, meropenem, fosfomicin, gentamicin, colistin	Deceased
9: male	67	Rectal cancer	3	27	Wound	–	Abscess	Gentamicin, tigecycline	Discharged

KPC-KP=*Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*; BAL = bronchoalveolar lavage

Table 2 Antibiotic susceptibilities minimum inhibitory concentrations [MICs], mg/L) of KPC-2-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates as determined by the Vitek 2 System

Antibiotic	Patient										
	1		2	3	4	5		6	7	8	9
	<i>K. pneumoniae</i>	<i>E. coli</i>				Isolate 1	Isolate 2				
Ertapenem	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8
Imipenem	4	2	4	4	≥16	2	4	4	8	4	4
Meropenem	2	1	2	2	8	≥16	≥2	2	2	1	2
Ampicillin	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32	≥32
Cefotaxime	16	4	16	16	≥64	16	16	≥64	16	16	16
Ceftazidime	≥64	4	≥64	≥64	≥64	≥64	≥64	≥64	≥64	≥64	≥64
Cefpodoxime	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8
Piperacillin	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128
Piperacillin/tazobactam	≥128	64	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128	≥128
Ciprofloxacin	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4
Moxifloxacin	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8	≥8
Gentamicin	4	≥16	4	4	4	4	4	4	2	4	4
Tobramycin	≥16	8	≥16	≥16	≥16	≥16	≥16	≥16	≥16	≥16	≥16
Tetracycline	4	≥16	4	4	4	8	8	4	4	4	4
Tigecycline	2	≤0.5	2	2	2	4	4	1	2	2	2
Cotrimoxazole	≥320	≥320	≥320	≥320	≥320	≥320	≥320	≥320	≥320	≥320	≥320
Colistin (E-test)	0.75	0.5	1.5	1.5	1.5	3	2	0.5	1.5	3.0	0.25

[37]. By use of a non-radioactively labelled *bla*_{KPC} PCR fragment, the plasmids showed the presence of *bla*_{KPC} DNA sequences in Southern hybridisation experiments (data not shown). Southern blotting of plasmid preparations with a biotin-labelled *bla*_{KPC-2} amplicon as a probe revealed a size larger than the plasmid pMG 252 (data not shown).

A *bla*_{KPC}-positive plasmid could be successfully mobilised from a *K. pneumoniae* and the *E. coli* isolate into an *E. coli* J53 recipient with a conjugation frequency of 2–3 × 10⁻⁸. Accordingly, the transconjugant showed a three to larger than 11-fold increase of the MIC as compared to the susceptibility of the recipient in all beta-lactams, cephalosporins and carbapenems tested. No such changes occurred when testing ciprofloxacin, gentamicin, tetracycline and trimethoprim-sulfamethoxazole (Table 3). Of note, in the presence of clavulanic acid, the MIC of all antimicrobials tested decreased by up to more than six dilution steps.

Sequence analysis of the three variable sites of Tn4401 elements described in the literature revealed data identical to plasmid pNYC (GenBank accession number EU176011) for both the clinical *E. coli* and *K. pneumoniae* isolate, as well as both resulting transconjugants. The left and right target site duplication with the particular sequence ATTGA was demonstrated by sequencing positions 3969–4398 and 13279–13979 (numbering according to GenBank accession

number EU176011). Furthermore, the determination of transposon sequences at positions 10827–11263 revealed the 100-bp deletion upstream of *bla*_{KPC-2}.

Discussion

To the best of our knowledge, this is the first outbreak of a KPC-producing *K. pneumoniae* in Middle Europe. Although cases from other Middle European countries have been reported, all of these were single cases and transmission was not observed [7, 17]. The characteristics of our outbreak—long hospitalisation periods of the respective cases, predominantly respiratory colonisation, high proportion of infected patients and a high overall mortality of infected patients—were similar to other outbreaks described so far.

In an outbreak described by Woodford et al., 24 ICU patients were colonised or infected with KPC-producing *K. pneumoniae* [6]. All infections were nosocomially acquired, with the patients having been hospitalised from 9 to 374 days prior to isolation of the organism. The organisms were isolated predominantly from respiratory secretions, but also from urine and blood. A high proportion, i.e. 14 of the 24 patients, was infected and eight of the infected patients died.

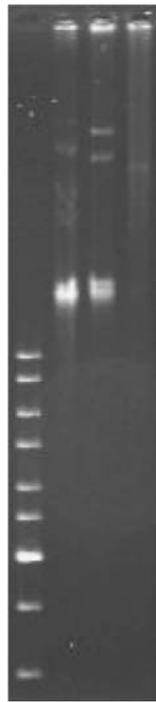


Fig. 2 Agarose gel of plasmid preparations from *bla*_{KPC-2}-positive isolates. From left to right: marker, *K. pneumoniae* patient 1, *E. coli* patient 1, *E. coli* J53 bearing pMG252

Bratu et al. described outbreaks in two New York hospitals involving 58 patients [38]. Forty-three patients were considered to have nosocomial infections. The outcome of patients with bacteraemia showed an overall 14-day mortality of 47%.

The outcome of our patients seemed to be even worse compared to these data. As the isolates were broadly resistant to most antibiotic classes, various combination therapies were used with little effect. Although the isolates showed susceptibility to intermediate MICs to tigecycline as described previously [39], the clinical efficacy seemed to be low. One isolate became resistant to tigecycline (MIC >8 µg/ml) during therapy.

The source of the KPC-producing *K. pneumoniae* could not be fully elucidated, although two facts suggest that the strain has been imported from Greece: the isolation of a KPC-producing *K. pneumoniae* from a Greek patient with a PFGE pattern that was identical to most strains that have been isolated during the outbreak. In addition, the transposon structure, including the target site duplication sequence and a 100-bp deletion, was identical to two previously reported plasmids in *K. pneumoniae* isolates from France and Greece. Of interest, these plasmids were 80 kb in size, which are considerably smaller than the *bla*_{KPC-2}-harbour-

Table 3 Antimicrobial susceptibility determined for donor, recipient and transconjugant by microbroth dilution testing according to the NCCLS/CLSI

Antibiotic	Recipient, donor and transconjugant strains				
	<i>E. coli</i> J 53	Patient 1 <i>K. pneumoniae</i>	conj. #104	Patient 1 <i>E. coli</i>	conj. #99
Amoxicillin	8	>256	>256	>256	>256
Amoxicillin+CLA	8	>256	256	>256	>256
Piperacillin	2	>128	>128	>128	>128
Piperacillin+TZB	2	>256	>256	>256	256
Cefoxitin	4	128	32	32	16
Cefotaxime	0.12	>256	>256	>256	>256
Cefotaxime+CLA	0.06	16	4	16	4
Ceftazidime	0.5	>128	32	32	32
Ceftazidime+CLA	0.25	128	4	32	4
Cefepime	0.06	>256	16	>256	64
Imipenem	0.25	128	16	32	16
Imipenem+CLA	0.125	16	2	8	1
Meropenem	0.03	32	8	16	8
Meropenem+CLA	<0.015	8	0.125	8	0.5
Ertapenem	<0.008	64	8	32	8
Ertapenem+CLA	<0.008	32	4	8	2
Ciprofloxacin	<0.015	128	<0.015	128	<0.015
Gentamicin	0.25	2	0.25	0.25	0.25
Tetracycline	1	4	1	256	256
Trimethoprim-sulfamethoxazole	0.03/0.57	128/2432	0.06/1.14	128/2432	128/2432

CLA = clavulanic acid; TZB = tazobactam

ing plasmids described here, and which may indicate recombination events.

In most incidents of isolation of KPC-producing *K. pneumoniae* in European countries, a link could be established to countries where occurrence of KPC had been reported previously. Naas et al. reported that the KPC-2 identified in France likely resulted from an intercontinental transfer of the KPC producer from the United States [7], whereas at least one of the two recently described *K. pneumoniae* isolates with KPC-3 carbapenemase detected in the United Kingdom was probably imported into the United Kingdom from Israel [17]. Two KPC-2-producing *K. pneumoniae* isolates were exported from Greece; one to France [14] and the other to Sweden [16].

As the rapid spread of KPC has been reported from the United States [1] and Israel [12], we hoped to contain further spreading of KPC carbapenemase. A common source for the outbreak could not be established and the increasing risk of transmission with increasing contact times suggests that transmission via the hands of health care workers was the most likely mechanism of spread. With regard to the high pathogenicity of the organisms and the reported experiences with prolonged outbreaks [3, 6, 12], we decided to implement strict isolation of the cases for the whole period of hospitalisation.

In addition, we searched for asymptomatic carriers using two prevalence screenings in our institutions. Because no screening method was established in our laboratory, we decided to rely on a non-selective medium and a medium selective for ESBLs. We decided to test swabs from the throat and wounds, and rectal swabs, because these body sites were frequently positive in cases. The sensitivity of swabs taken from different body sites to detect carriers of KPC-producing *K. pneumoniae* has not been described so far. Thus, we cannot assess the risk of missing carriers. However, all known carriers who were tested by this screening method had positive results.

Although we may have terminated the outbreak in our institution, the high number of contact patients that resulted from long hospitalisation times and frequent transferring of the highly care-dependant patients makes it unlikely that the establishment of KPC in Germany could be prevented.

MICs derived by the Vitek 2 system were usually comparably low for imipenem and meropenem, whereas testing with microbroth dilution revealed higher MICs. This problem has been reported previously [40]. However, the Vitek 2 system is widely used in German laboratories and, if included in the testing panel, ertapenem may serve as an indicator for the detection of KPC carbapenemases. The low in vitro efficacy of ertapenem in comparison to other carbapenems remains to be elucidated.

In conclusion, KPC-producing *K. pneumoniae* has been introduced to our university hospital, possibly from

Greece, and was probably further transmitted by the hands of health care workers. The high number of contact patients to whom transmission may have occurred makes it likely that the KPC enzyme has established in Germany and probably in Europe. Thus, suspicion must be high in multi-resistant enterobacterial isolates with elevated MICs to ertapenem.

Potential conflicts of interest C.W. is a speaker for Pfizer and BD Diagnostics; M.A.W. is a speaker for Wyeth, Pfizer, MSD, Essex, Gilead, Astra Zeneca and Astellas. All other authors have no conflict of interest to declare.

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