Emergence of four cases of KPC-2 and KPC-3-carrying *Klebsiella pneumoniae* introduced to Switzerland, 2009–10

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We report four epidemiologically unrelated cases of KPC-carrying *Klebsiella pneumoniae* identified in Switzerland between May 2009 and November 2010. Three cases were transferred from Italy (two KPC-3, one KPC-2) and one from Greece (KPC-2). Resistance to colistin and doxycycline emerged in one KPC-3-carrying *K. pneumoniae* strain during therapy. These results demonstrate ongoing dissemination of KPC throughout Europe. Rapid and reliable identification of KPC and implementation of control measures is essential to limit spread.

Introduction

Carbapenems are first-line drugs for severe infections caused by *Enterobacteriaceae* expressing extended-spectrum beta-lactamases (ESBLs). The emergence of carbapenemase-producing *Enterobacteriaceae* in the past years is of great concern [1]. After the characterisation of the first *Klebsiella pneumoniae* isolate producing carbapenemase of the KPC type in 1996 in the United States [2], the KPC-producing bacteria spread worldwide. Poirel et al. reported the first KPC-2 *K. pneumoniae* isolate in Switzerland imported from Sicily (Italy) in 2010 [3]. Here, we report four additional imported cases of KPC-carrying *K. pneumoniae* detected in Switzerland.

Materials and methods

The four KPC-suspected strains were collected from different Swiss hospitals and were sent to our laboratory in Basel for confirmation. We performed conventional susceptibility testing using an automated micro-dilution test system (Micronaut-S, MERLIN Diagnostika mbH). Determination of minimum inhibitory concentrations (MIC) was performed with Etest stripes (bioMérieux). Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [4], except for doxycycline

which was interpreted according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [5].

For phenotypic identification of KPC-producing isolates, both double disc synergy test and modified Hodge test were applied [1]. KPC-specific PCR and direct sequencing of the region encompassing the main part of the *bla*_{KPC} gene (ca. 820 nucleotides, sequenced in both directions) was performed according to Bradford et al. [6]. The KPC type was identified by the BLAST programme from the National Center for Biotechnology Information Web site (<http://www.ncbi.nlm.nih.gov/BLAST>).

Clinical data were available for two patients and were collected by full chart review.

Results

Four cases of KPC-carrying *K. pneumoniae* were detected in Switzerland between May 2009 and November 2010, two of type KPC-2 and two of type KPC-3. All patients were between 50 and 70 years-old and had been transferred from abroad, three from Italy and one from Greece. Two patients were colonised and two were infected with KPC-producing *K. pneumoniae* (Table 1).

The antimicrobial susceptibility profiles of the four *K. pneumoniae* isolates are listed in Table 2. Double disc synergy test as well as the modified Hodge test showed positive results for KPC-production in all four cases.

Case 1

The patient in their 50s lived in Greece and travelled to Switzerland in September 2009. The person was colonised in the urine, inguinal and perineum with

K. pneumoniae bla_{KPC-2}. No further clinical data were available for this patient.

Case 2

The patient was in their 60s and hospitalised in Italy due to upper gastro-intestinal problems, and underwent diagnostic endoscopy. The clinical history did not mention any antibiotic treatment. In May 2010 the

patient was transferred to Switzerland. On hospital admission a constricted bile duct was stented endoscopically. Because the patient developed fever during the initial empiric antibiotic therapy with piperacillin/tazobactam, the therapy was changed to meropenem. During treatment with meropenem the patient developed bloodstream infection with *K. pneumoniae*. The isolate was resistant or intermediately resistant to all

TABLE 1

Main characteristics of four cases of KPC-producing *K. pneumoniae* detected in Switzerland, May 2009 and November 2010

	Case 1	Case 2	Case 3	Case 4
Age group (years)	50-59	60-69	40-49	60-69
Date of KPC detection	September 2009	July 2010	September 2010	November 2010
Infected/colonised with <i>K. pneumoniae</i>	Colonised	Infected	Infected	Colonised
Transferred from	Greece	Italy ^a	Italy ^a	Italy ^a
Hospitalised in Switzerland	Eastern part	North-western part	Western part	North-eastern part
bla _{KPC} gene	bla _{KPC-2}	bla _{KPC-3}	bla _{KPC-2}	bla _{KPC-3}

KPC: *Klebsiella pneumoniae* carbapenemase.

^a The three patients were transferred from three different regions in Italy: Apulia, Liguria and Sicily

TABLE 2

Antimicrobial susceptibility profiles of the four *Klebsiella pneumoniae* first isolates carrying bla_{KPC-2} or bla_{KPC-3}, Switzerland, May 2009 and November 2010

Antibiotic	Case 1 bla _{KPC-2}	Case 2 bla _{KPC-3}	Case 3 bla _{KPC-2}	Case 4 bla _{KPC-3}
Ampicillin	R	R	R	R
Amoxicillin/clavulanate	R	R	R	R
Piperacillin/tazobactam	R	R	R	R
Cefuroxime	R	R	R	R
Ceftazidime	R	R	R	R
Ceftriaxone	R	R	R	R
Cefepime	R	R	R	R
Aztreonam	R	R	R	R
Imipenem	R (≥32)	R (≥32)	R (16)	R (≥32)
Meropenem	R (≥32)	R (≥32)	R (12)	R (≥32)
Ertapenem	R (≥32)	R (≥32)	R (16)	R (≥32)
Trimethoprim/sulfamethoxazole	R	R	R	R
Nitrofurantoin	R	R	ND	R
Norfloxacin	R	R	ND	R
Ciprofloxacin	R (≥32)	R (≥32)	R (≥32)	R (≥32)
Levofloxacin	R	R	ND	R
Gentamicin	S (1)	S (2)	S (2)	I (4)
Tobramycin	ND	I	R	ND
Amikacin	S	S	R	ND
Netilmicin	R	R	R	ND
Colistin	R (4)	S (0.25) ^a R (16) ^b	S (0.25)	S (0.5)
Doxycycline	S (4)	S (4) ^a R (16) ^b	I (8)	I (8)
Tigecycline	S (1)	I (2)	S (1)	I (2)
Fosfomycin	S (32)	R (128)	S (32)	R (128)

I: intermediate; ND: no data; R: resistant; S: susceptible.

Minimum inhibitory concentration in mg/L is given in parentheses.

^a Before antimicrobial treatment.

^b After antimicrobial treatment.

antibiotics tested (see Table 2) except gentamicin, colistin, and doxycycline. KPC-specific PCR was performed detecting *bla*_{KPC-3}. The antibiotic therapy was adapted to intravenous colistin and gentamicin. Repeated stenosis of the intra-ductal stent and suspicion of cholangiocarcinoma provided evidence for a Whipple operation with pancreaticogastrostomy. After the operation the patient developed acute arterial bleeding and had to be surgically revised. Shortly after the second operation he had relapsing bloodstream infection with *K. pneumoniae* which were newly resistant to colistin. Colistin treatment was stopped and doxycycline and later fosfomycin were added to gentamicin. Fosfomycin was given because routine testing revealed an intermediate result. The patient died from multiorgan failure caused by an uncontrolled infection complicated by intra-abdominal bleeding. A bronchoalveolar lavage specimen taken one day before death was positive for *K. pneumoniae*, now also resistant to doxycycline.

Case 3

The patient in their 40s was injured in July 2009 in Italy. The person was treated initially with an external fixation for a pelvic fracture in a primary care hospital in Italy and referred to the University Hospital of Berne for secondary care. The surgeon noted a pin track infection and performed a wound excision. Perioperatively, as well as at discharge, no signs of infection were present. In September 2010 the patient was readmitted because of a pelvic abscess, which was drained and osteosynthesis material was removed. Empiric therapy included amoxicillin/clavulanate, but cultures of the abscess revealed a *K. pneumoniae* isolate resistant to all antibiotics tested except gentamicin, colistin, tigecycline and fosfomycin (Table 2).

Antibiotic treatment was switched to tigecycline and gentamicin, followed by a second and third surgical debridement. After two weeks gentamicin was discontinued because of ototoxicity and therapy with tigecycline for a chronic osteomyelitis was continued for three months. To the best of our knowledge the patient was cured with this antibiotic course.

Case 4

The patient was in their 60s and hospitalised in an intensive care unit in Italy for one month because of a cerebral vascular insult, before being transferred to Switzerland. This patient was colonised in the upper respiratory tract and on the subclavian catheter with *K. pneumoniae* that contained the *bla*_{KPC-3} gene.

Infection control measures

Cases 2 and 3 were isolated immediately after detection of KPC-producing *K. pneumoniae* by contact isolation [5,6], which involved stay in a single-patient room, use of gloves and gowns by medical personnel during physical contact, and use of masks by medical personnel when exposure to respiratory secretions was expected. Secondary spread was investigated by active surveillance. This included screening of all

patients sharing the same room as the index patient for ESBL or KPC by testing stool samples and, if applicable, samples from wounds, drainages, etc. None of these patients was found to be positive for KPC-carrying *Enterobacteriaceae*. Patients who stayed in the same room as the index patient and who were already discharged from hospital were tagged in the hospital administration system to be screened at the next admission. Infection control measures taken around Cases 1 and 4 could not be made available during this study.

Discussion and conclusions

The global dissemination of KPC-producing *K. pneumoniae* is of great concern to public health services worldwide [9]. The first outbreak outside the United States was documented in Israel in 2004 [10]. Several reports of outbreaks and sporadic cases of KPC-producing *K. pneumoniae* in Italy and Greece have been described and indicated rapid spread within these two countries [11-15]. On the epidemiological scale for spread of KPC-carrying *K. pneumoniae* defined by Grundmann et al. [1] Greece was determined in November 2010 as 'endemic' and Italy as a country with 'interregional spread'. The first case of a KPC-2-producing *K. pneumoniae* in Switzerland was described in 2010 from a patient transferred from Sicily [3].

We document four further cases of KPC-carrying *K. pneumoniae* introduced to Switzerland, three from Italy and one from Greece. Three patients have been discharged from hospital in a stable state of health, one patient died from uncontrolled infection complicated by arterial intraabdominal bleeding. In one case the KPC-3 carrying *K. pneumoniae* strain developed resistance to colistin and doxycycline despite combined antibiotic treatment.

Phenotypic and molecular tests for KPC detection were concordant and confirmed carbapenemase production in all strains by conventional susceptibility testing. As shown in Table 2, the antibiotic susceptibility patterns of the four independent isolates were similar.

In this study, we confirm continuous dissemination of KPC-producing isolates from Greece and Italy to Switzerland. The fact that the three patients transferred from Italy had been hospitalised in three different geographic regions in Italy, Apulia, Liguria and Sicily, is a further indication for a wider dissemination of these isolates. We found no evidence for local spread within the Swiss hospitals where the four reported cases were treated, but sporadic cases are thought to precede endemicity, a likely scenario for resistance markers encoded on plasmids [9]. The worldwide spread of KPC-carrying *K. pneumoniae* is worrying, since this species is a main source for hospital-acquired infections in critically ill patients and well known for its ability to transfer resistance determinants. Due to the high mobility of KPC genes we anticipate that KPC-mediated resistance will be a prominent mechanism of multidrug

resistance in Gram-negative bacilli in the near future. As antimicrobial treatment options are dramatically restricted, rapid and reliable identification of KPC is mandatory to generate concepts to limit spread.

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