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Ongoing rubella outbreak among adolescents in Salaj, Romania, September 2011–January 2012

D Janta (denisa.janta@insp.gov.ro)[1,2], A Stanescu[1], E Lupulescu[3], G Molnar[4], A Pistol[1]

1. National Institute of Public Health, National Centre for Communicable Diseases Surveillance and Control, Bucharest, Romania
2. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
3. National Institute of Research and Development for Microbiology and Immunology 'Cantacuzino' – Reference Laboratory for Measles and Rubella, Bucharest, Romania
4. Ministry of Health, Bucharest, Romania

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A rubella outbreak has been ongoing in Salaj, Romania since September 2011 involving 1,840 probable and confirmed cases among mainly unvaccinated adolescents. The index case had onset of illness on 6 September 2011. The highest number of cases was recorded among 10–14-year-olds and 15–19-year-olds. Complications were recorded for 11 cases and included meningitis and arthritis. Although the peak has passed, surveillance is being maintained in the region.

An outbreak of rubella with more than 1,800 cases was identified in Salaj, north-western Romania, a district with 241,014 inhabitants according to 2010 data (Figure 1).

The European Union (EU) case definition [1] was used in the outbreak investigation and of the 1,873 possible cases, 69 (3.6%) were classified as laboratory-confirmed, 1,771 (94.6%) as probable, defined according to clinical criteria and epidemiological links with a confirmed case, and 33 (1.8%) were discarded. Of the 69 laboratory-confirmed cases, two were pregnant women but no case of congenital rubella infection (CRI) has been reported so far.

Figure 1

District of Salaj, Romania

Background

Rubella is a statutorily notifiable disease in Romania since 1978 [2]. Until 2010, data were reported in aggregated format by age group. In 2010, a case-based reporting with mandatory laboratory confirmation was introduced.

Since December 2002, as part of the measles surveillance system, clusters of febrile rash are investigated by the local public health authorities and, in order to confirm the clinical diagnosis, it is recommended to take samples from five to ten cases in each cluster for serological testing for measles and, if the results are negative, for rubella. If rubella transmission is confirmed, pregnant women who are epidemiologically linked to a laboratory-confirmed case or who meet the clinical criteria are given priority for testing and are informed about the potential risks of congenital rubella syndrome (CRS) to the foetus. Although rubella is usually a mild febrile rash illness, infection during pregnancy can lead to miscarriage, stillbirth, and birth defects associated with CRS such as heart disease, blindness, deafness and mental retardation.

A national surveillance system for CRS was initiated in Romania in 2000. Newborns of rubella positive mothers have a rubella-specific IgM blood test tested for CRI and are reported in the CRI/CRS surveillance system according to the methodology in place [3].
Rubella is a vaccine preventable disease targeted for elimination in the WHO European Region by 2015 along with measles. Countries in the region have also committed to the prevention of CRS by the same year [4].

A rubella-containing vaccine was first available in Romania in 1998 (bivalent measles-rubella) and it was offered to girls aged between 15 and 18 years (birth cohorts 1980–1983) as part of a mass vaccination campaign following a nation-wide measles outbreak [5]. After a large rubella outbreak in 2002 to 2003, a rubella-containing vaccine was offered to girls aged between 13 and 14 years until 2008 (birth cohort 1994). In 2004, the measles-mumps-rubella (MMR) vaccine was introduced in the national immunisation schedule for children aged 12–15 months. Since 2004, MMR vaccination has also been offered to children aged seven years.

In 2002 to 2003, a large rubella outbreak occurred in Romania, with more than 115,000 cases reported nationwide and the highest incidence was reported in children of school age (2,564 per 100,000 population aged 5–9 years and 2,446 per 100,000 population aged 10–14 years [6]. By 2010, rubella incidence dropped to 1.6 per 100,000 population (2009: 2.9/100,000; 2008: 8.1/100,000) [7,8].

Outbreak description

The index case (laboratory-confirmed) was reported on 6 September 2011 to Salaj Public Health Authority in an unvaccinated 16-year-old student attending a local high school. Between 1 September 2011 and 23 January 2012, 1,840 confirmed and probable rubella cases were reported by Salaj Public Health Authority (Figure 2).

Of the 1,840 cases 1,069 were male and 771 were female, showing a male:female ratio of 1.4:1. The highest number of cases was recorded in people aged between10 and 19 years (n=1,693). Of these, 1,206 cases were registered among 15–19-year-olds (58.6% male and 41.4% female) with a male:female ratio of 1.4:1. The rest of 487 cases were registered among 10–14-year-olds (55.4% male and 44.6% female) with a male:female ratio of 1.2:1.Taking into account the fertile age, we registered 1,341 cases in people aged between 15 and 44 years with a male:female ratio of 1.3:1. Among these 1,341 cases 59.1% were male and 40.9% were female giving a male:female ratio of 1.3:1. Of all outbreak cases 23.3% (428/1,840) were registered among children born in 1996.

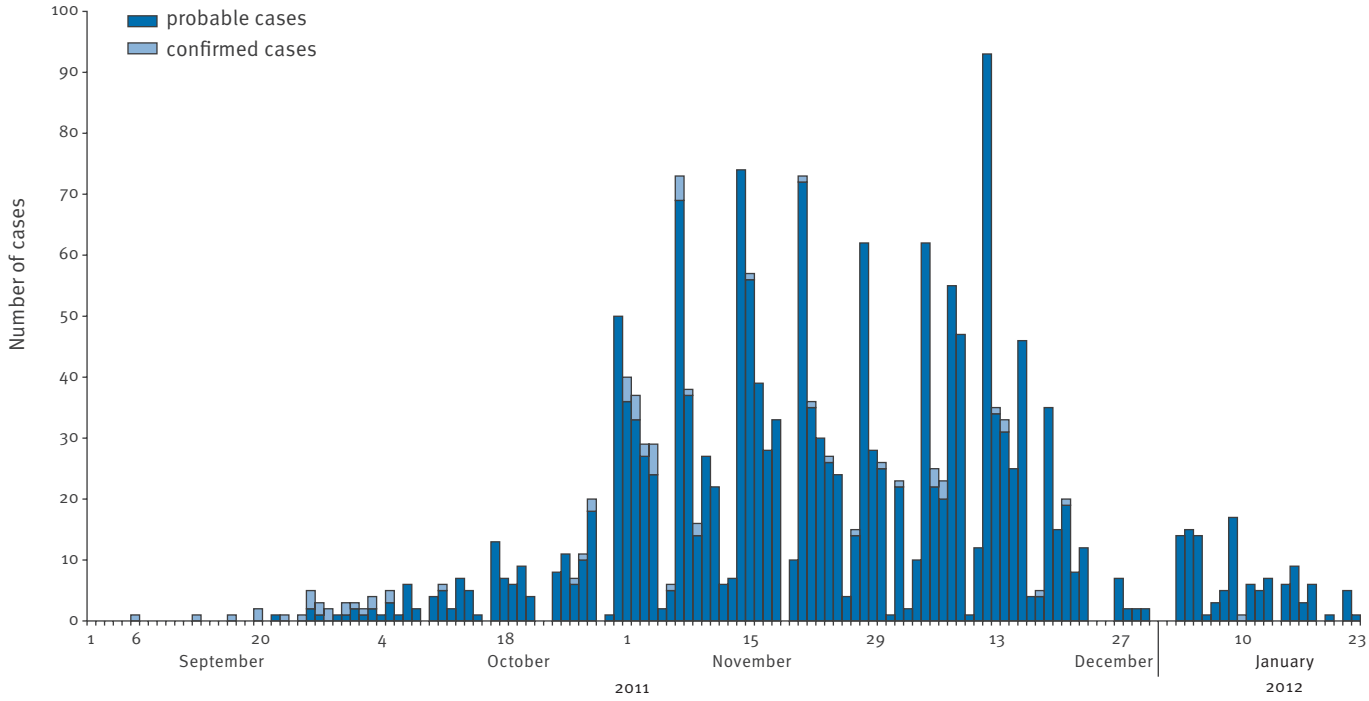
The incidence of rubella in Salaj was 763 per 100,000 population with the highest incidence among high school teenagers aged between 15 and 19 years (9,555 per 100,000 population for males and 7,067 for females) followed by 10–14 year-olds (3,854 per 100,000 population for males, 3,281 per 100,000 population for females).The third most affected age group was the age group of 20–24 year-olds with an incidence of 647 per 100,000 population among males and 154 per 100,000 population among females (Table).

Complications included meningitis (n=2 cases) and arthritis (n=9 cases). Thirty-five cases required hospitalisation and the median length of hospital stay was four days (minimum 1, maximum 9).

A total of 98 samples were tested for rubella IgM antibodies and 69 of these were confirmed as positive.

Figure 2

Distribution of probable and confirmed rubella cases by date of symptom onset, Salaj, Romania, 1 September 2011–23 January 2012 (n=1,840)



In clinical specimens from two cases rubella virus genotype 2B was identified.

Vaccination coverage among the reported cases was low: 38 (2.1%) of the total number of cases were vaccinated with one dose of rubella-containing vaccine. Taking into account the eligible birth cohorts for vaccination against rubella and the number of rubella cases reported during the outbreak, we calculated the proportion of rubella cases occurring in birth cohorts from 1989 to 2002. The highest proportion of cases was registered among people born from 1994 to 1997 (Figure 3).

We looked at the rubella vaccination coverage for Romania and Salaj district in the eligible birth cohorts for MMR vaccination at 12–15 months (birth cohort 2004–2008), for MMR vaccination at seven years (birth cohort 1998–2002) and the rubella dose administered only to girls in birth cohort 1989–2003. The rubella vaccination coverage of the 13 to 14 year-old females in Salaj District during 2003 to 2008 is higher with 3.8% on average than the coverage in the whole country for the same period. For 2005 to 2009, the MMR vaccination coverage in Salaj was below the country average, except for seven-year-old children vaccinated with MMR in 2008 and 2010 when the district coverage was higher than the country average (Figure 4).

Table

Number of probable and confirmed cases (n=1,840) and incidence of rubella by sex and age group, Salaj, Romania, 1 September 2011–23 January 2012

Age group (years)	Male		Female	
	Number of cases	Incidence per 100,000 population	Number of cases	Incidence per 100,000 population
0-4	4	62	4	64
5-9	3	46	2	31
10-14	270	3,854	217	3,281
15-19	707	9,555	499	7,067
20-24	64	647	14	154
25-29	10	112	3	36
30-34	4	42	12	139
35-39	4	42	11	124
40-44	3	31	9	100
Total	**1,069**	**1,429**	**771**	**1,097**

Figure 3

Proportion of rubella cases by birth cohort, Salaj, Romania, 1 September 2011–23 January 2012

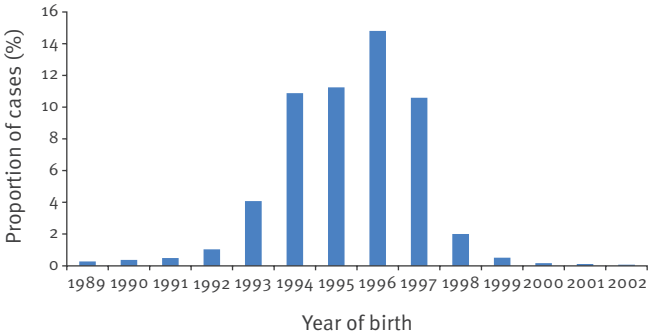
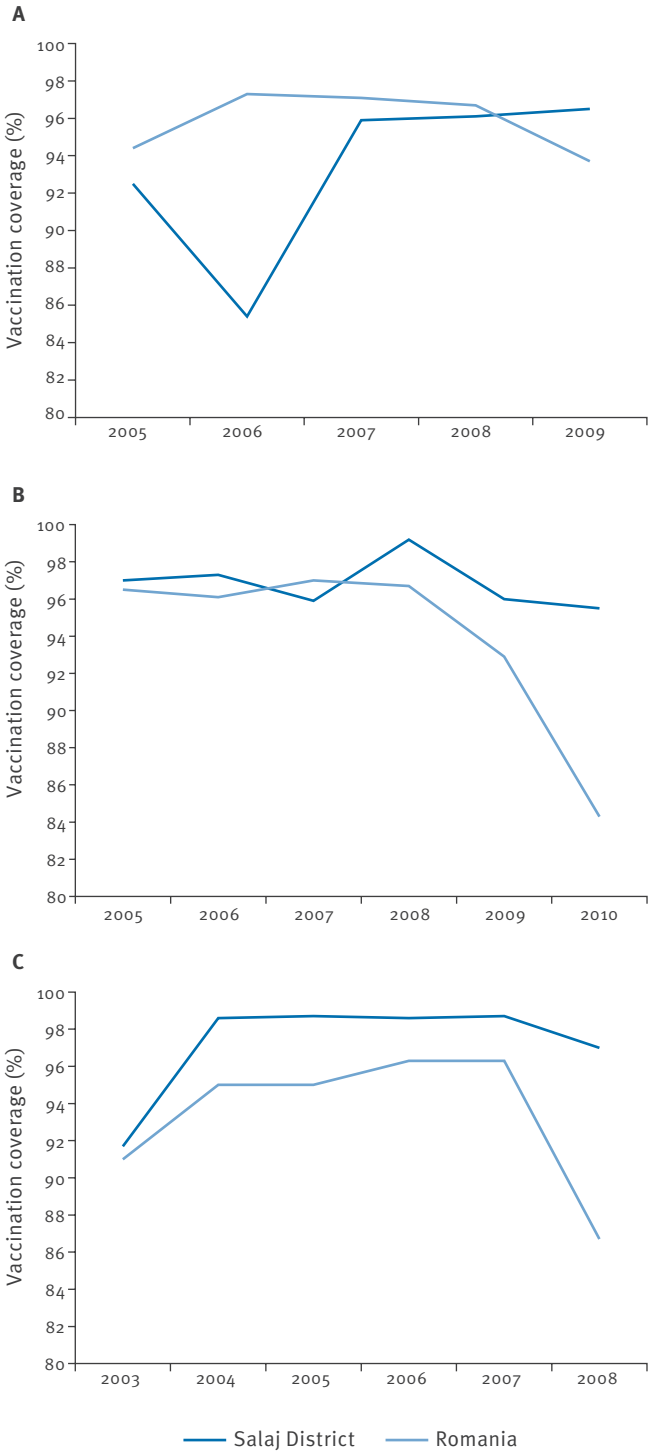


Figure 4

Coverage of (A) MMR vaccination at 12–15 months (2005–2009), (B) MMR vaccination at seven years (2005–2010) and (C) rubella-containing vaccine in girls aged 13–14 years (2003–2008), Romania and Salaj district



Control measures

Since the identification of the outbreak, several control measures have been implemented by the local health authorities. The most important one was the initiation of a MMR vaccination campaign in the district of Salaj targeting all children and adolescents aged between 10 and 19 years, irrespective of their vaccination status. The MMR vaccine is supplied by the Ministry of Health and is offered free of charge through routine immunisation services (family physicians) and special outreach teams. As a result of this special campaign, 210 persons were vaccinated until 31 December 2011 but many parents still refused to have their children vaccinated. Local healthcare workers (doctors and nurses) were recommended to get vaccinated and to try to inform and increase awareness among their patients on the risk of this disease, especially among women of childbearing age.

Additionally, the local public health authorities have initiated rubella screening among pregnant women epidemiologically linked to a probable or a confirmed case. While the general recommendations for MMR vaccination are maintained at national level, some districts bordering Salaj (Alba, Bihor and Cluj) – which were also affected by rubella but to a lesser extent – have issued vaccination recommendations among teenagers to avoid other potential outbreaks.

Discussion and conclusions

The results of the investigation revealed that of the total number of rubella described above, 98% had never been vaccinated against rubella infection. The index case was an unvaccinated teenager and 1,206 of the rubella cases (65.5%) occurred among teenagers aged between 15 and 19 years (born between 1992 and 1996). Taking into account the historical MMR vaccination schedule in Romania, 770 children born between 1995 and 1996 (15-16-year-olds in 2011) were not eligible for rubella vaccination and represent 41.8% of all cases. In the birth cohort 1992–1994 only girls were eligible of rubella vaccine at the age of 14 years.

Measures such as catch up campaigns are important to close existing gaps in vaccination and prevent further spread of the outbreak. Educating the general public on modes of rubella transmission and stressing the need for rubella vaccination is the most important way to prevent further spread of the disease in other districts and to prevent congenital infection.

Although the outbreak has passed its peak and is subsiding, surveillance for rubella will be maintained for a least two incubation periods (46 days) following onset of rash of the last case [9]. Moreover, active surveillance for infants with CRS will be carried out until the age of nine months after the last reported rubella case [10].

Acknowledgments

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Isolation of NDM-1-producing *Klebsiella pnemoniae* in Ireland, July 2011

H McDermott[1], D Morris[2], E McArdle[3], G O'Mahony[1], S Kelly[1], M Cormican[2], R Cunney (robert.cunney@hse.ie)[1]
1. Department of Clinical Microbiology, Children's University Hospital, Temple Street, Dublin, Ireland
2. Antimicrobial Resistance and Microbial Ecology Group, National University of Ireland, Galway, Ireland
3. Department of Public Health, Health Services Executive, Dr Steeven's Hospital, Dublin, Ireland

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We report the identification of New Delhi metallo-beta-lactamase 1 (NDM-1)-producing *Klebsiella pnemoniae* in Ireland. The organism was resistant to multiple antibiotic classes, including carbapenems, and PCR and sequencing confirmed the presence of the bla_{NDM-1} gene, carried on a 98kb plasmid. The organism was isolated from an infant, who was born in India and moved to Ireland at the age of four months. This is the first reported isolation of an NDM-1-producing *Enterobacteriaceae* strain in Ireland.

Case report

A six-month old infant presented to the family doctor in May 2011 with a non-specific febrile illness. The child had been born in Kolkata, eastern India, by uncomplicated full-term vaginal delivery. The mother and child spent three to four days in hospital after the birth. The family (two parents and child) moved to Ireland when the child was four months old. The child, who had no underlying illness or past medical history of note, was treated empirically with amoxicillin/clavulanic acid for a suspected urinary tract infection shortly after arrival in Ireland; however, no urine sample was submitted for testing at this time. Six weeks later, the child presented again with a low-grade fever. A urine sample showed a white cell count of 1,700/mm^3 and greater than 10^5 bacteria/ml on culture. The isolate was identified as *Klebsiella pneumoniae* on VITEK 2 (bioMérieux, United States). It was resistant to meropenem (minimum inhibitory concentration (MIC) >16 mg/L), but susceptible to ciprofloxacin. After treatment with a 10-day course of ciprofloxacin, there was a good clinical response. A repeat urine sample after completion of ciprofloxacin therapy grew more than 10^5 bacteria/ml of an isolate identified as *Escherichia coli* resistant to ciprofloxacin, cefotaxime and cefoxitin but susceptible to ertapenem and trimethoprim. The child received a five-day course of trimethoprim and remains clinically well. A renal ultrasound was normal.

A rectal swab from the child, taken two weeks after the initial positive urine sample, yielded multiple isolates of *K. pneumoniae* including both carbapenem-resistant and carbapenem-susceptible but extended-spectrum cephalosporin-resistant isolates.

The case was investigated by the local Department of Public Health in accordance with international protocols [1]. Family screening for carriage of the NDM-1-producing strain was carried out on both urine and rectal samples of the parents. *E. coli* with a similar susceptibility pattern to the second urinary isolate from the child was isolated from rectal swabs from the parents, but carbapenem-resistant *K. pneumoniae* was not detected.

Laboratory characterisation

The carbapenem-resistant *K. pneumoniae* isolate from the initial positive urine sample (isolate number 2661) was referred to the Antimicrobial Resistance and Microbial Ecology Group at the National University of Ireland, Galway, for further characterisation. Meropenem and ertapenem MICs were both >32 µg/ml as determined by Etest. The full susceptibility profile, as determined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method [2], is shown in the Table. The isolate was confirmed as a carbapenemase producer by the modified Hodge test of the CLSI, and metallo-beta-lactamase activity was indicated by a commercial synergy test (Rosco Diagnostica, Denmark). PCR and sequencing confirmed the presence of bla_{NDM-1}, and plasmid analysis revealed this was carried on a 98kb plasmid (data not shown) [3,4]. PFGE analysis using *Xba*I was carried out on two *K. pneumoniae* isolates from the child (the carbapenem-resistant *K. pneumoniae* and a carbapenem-sensitive *K. pneumoniae* rectal swab isolate) and on *E. coli* isolated from the child and from both parents [5]. The PFGE profiles of the two *K. pneumoniae* isolates from the child were not similar (data not shown). PFGE profiles of the three *E. coli* isolates were indistinguishable (one from each of the parents and one from the child).

Discussion

Infections caused by carbapenem-resistant *Enterobacteriaceae* isolates have been reported in

hospital outbreaks in Ireland [6], but isolates producing NDM-1 have not previously been identified in Ireland.

Carbapenemase-producing *Enterobacteriaceae* represent a major threat to current approaches to treatment of life-threatening *Enterobacteriaceae* infection. In addition to resistance to almost all available beta-lactam agents, many strains are frequently resistant to multiple classes of antimicrobial agents, including aminoglycosides and fluoroquinolones.

NDM-1-producing *K. pneumoniae* was first recognised in a Swedish patient in 2008 who was repatriated to Sweden from the Indian subcontinent [7]. Since then, NDM-1-producing isolates have been identified in patients in the United Kingdom who had a history of receiving healthcare in India and Pakistan [8]. The majority of reported clinical cases related to NDM-1-producing isolates to date have been in adults. However, NDM-1-producing *E. coli* has recently been reported from rectal screens of neonates returning to France after having attended healthcare facilities in Egypt and India [9]. Two cases of neonatal sepsis associated with NDM-1-positive *K. pneumoniae* have been reported from a neonatal intensive care unit in a tertiary referral hospital in Kolkata, India [10].

The source of colonisation/infection with NDM-1-producing *K. pnemoniae* in the child reported here cannot be established unequivocally. However, the fact that the child was born and lived the first few months in India, including a stay of a few days in hospital after birth, is likely to be of relevance given the reported high levels of carbapenemase-producing *Enterobacteriaceae* in India [11]. Although such organisms were not detected in either parent, a single rectal swab may not identify carriage, particularly if the organism is present in small numbers [12], and the parents may therefore potentially be colonised.

This case highlights the importance of testing isolates from routine clinical samples for susceptibility to carbapenem even in low-incidence areas to maximise the likelihood of detection of carbapenem-resistant *Enterobacteriaceae*, in order to guide therapy and prevent onward spread through implementation of transmission-based precautions and enhanced environmental cleaning (as was done in this case). Early recognition and reporting in low-incidence areas also provides an opportunity to establish national measures to prevent such isolates becoming endemic in healthcare settings. This report also highlights the importance of considering the possibility of carbapenem-resistant isolates in people returning from the Indian subcontinent.

Table

Susceptibility profile of NDM-1-producing *Klebsiella pneumoniae* urinary isolate recovered in Ireland, July 2011

Antibiotic	Susceptibility
Chloramphenicol	S
Minocycline	S
Tetracycline	S
Ciprofloxacin	S
Amikacin	R
Kanamycin	R
Ampicillin	R
Ceftazidime	R
Cefotaxime	R
Cefpodoxime	R
Cefoxitin	R
Aztreonam	R
Amoxicillin/clavulanic acid	R
Piperacillin/tazobactam	R
Sulphonamides	R
Streptomycin	R
Gentamicin	R
Nalidixic acid	R
Trimethoprim	R

NDM-1: New Delhi metallo-beta-lactamase 1; R: resistant; S: sensitive.

Susceptibility was determined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method [2].

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NDM-1 producing *Acinetobacter baumannii* isolated from a patient repatriated to the Czech Republic from Egypt, July 2011

J Hrabák (Jaroslav.Hrabak@lfp.cuni.cz)[1], M Štolbová[2], V Študentová[1], M Fridrichová[3], E Chudáčková[1], H Zemlickova[3]
1. Department of Microbiology, Faculty of Medicine in Plzeň, Charles University in Prague, Plzeň, Czech Republic
2. Department of Clinical Microbiology, Masaryk´s Hospital, Ústí nad Labem, Czech Republic
3. National Reference Laboratory for Antibiotics, National Institute of Health, Prague, Czech Republic

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We describe the isolation of an NDM-1-producing *Acinetobacter baumannii* in a Czech patient repatriated in July 2011 from Egypt. The infection spread to another patient on the same ward. Both isolates showed the same resistance pattern and were susceptible only to colistin. They had an identical PFGE pattern and belonged to the same sequence type ST 1. Sequencing of the bla_{NDM} gene identified the NDM-1 variant of the carbapenemase, surrounded by two copies of insertion sequence IS*Aba125*.

Here we describe the isolation of a New Delhi metallo-beta-lactamase-1 (NDM-1)-producing *Acinetobacter baumannii* in a Czech citizen repatriated from Egypt in July 2011. The patient was hospitalised in Egypt, and then transferred to a hospital in the Czech Republic. The patient developed ventilator-associated pneumonia caused by *A. baumannii* in addition to a primary neurological diagnosis. A carbapenem-resistant *A. baumannii* strain (V509) was isolated from bronchoalveolar lavage and an oral cavity swab. He was initially treated by meropenem and metronidazole. Due to progression of the primary disease, the patient was transferred to a long-term intensive care unit. Although the antibiotic regimen was not changed, the patient recovered according to the biochemical markers of inflammation within seven days and the antibiotic therapy was then stopped. The available data are not conclusive as to whether this patient was infected or colonised. However, the resistant isolate has been detected in low quantity in oral swab and bronchoalveolar lavage until the transfer to the long-term intensive care unit. The intensive care centre was informed about the epidemiological risk associated with this patient so that they could prepare for appropriate measures upon transfer.

A second *A. baumannii* isolate (V566) with the same resistance pattern was recovered six days later from the airways of another ventilated patient sharing the same room. The patient was treated with amoxicillin/clavulanic acid, chloramphenicol and ciprofloxacin. He died due to respiratory failure four days after the first isolation of NDM-1-producing *A. baumannii*.

Laboratory analysis

The isolates from both patients were identified as *A. baumannii* by biochemical test API ID32 GN (bioMérieux, France) and by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Germany). The minimum inhibitory concentrations (MICs) to 14 antibiotics were tested and the results were interpreted according to the EUCAST recommendation [1]. The isolates from both patients were resistant to all beta-lactams tested including carbapenems and other antibiotics (Table).

Typing performed by pulsed-field gel electrophoresis (PFGE) [2] showed that the isolates had indistinguishable macrorestriction patterns. Carbapenemase production was confirmed by MALDI-TOF mass spectrometry [3]. Production of metallo-beta-lactamase (MBL) activity was verified by ethylenediaminetetraacetic acid (EDTA) double-disk synergy test [4].

The bla_{NDM} gene of both isolates was amplified and sequenced as described previously [5], and revealed the NDM-1 variant of the enzyme. The bla_{NDM-1} together with other genes was located between two copies of the insertion sequence IS*Aba125* in the same orientation as found by Pfeifer et al. [6]. Because plasmid preparations from the two isolates did not yield any plasmids visible after electrophoretic separation, and no transformants were obtained after transformation experiments performed as previously described [7], it can be hypothesised that bla_{NDM-1} is located on the bacterial chromosome.

Multi-locus sequence typing (MLST) was performed [8] and the MLST database available at the website of the Pasteur Institute was used to assign the sequence type (ST). Both isolates belonged to sequence type (ST) 1 (allelic profile 1-1-1-1-5-1-1) which represents the epidemiologically successful European clone I [9].

Discussion and conclusion

Reports describing NDM-type carbapenemase producers isolated from patients previously hospitalised in high-prevalence countries have been increasing. Pfeifer et al. detected NDM-1 in *A. baumannii* isolated from a patient repatriated to Germany from Serbia in 2007 [6]. Importation of NDM-1-producing *A. baumannii* strain from Serbia has also been described by Poirel et al. [10]. Other *A. baumannii* isolates expressing NDM-1 MBL have been isolated in China and India [11,12]. It is remarkable that bla_{NDM-1} was also found on a plasmid in *A. lwoffii* in China [13]. The new NDM-2 variant was first detected in *A. baumannii* from a patient transferred from Egypt to Germany [5]. Recently, clonal spread of NDM-2-producing *A. baumannii* strains have been described in a rehabilitation ward in Israel and in the United Arab Emirates [14,15].

Until this report, no NDM-1 producing bacterium had been described in the Czech Republic, a country with a low prevalence of carbapenemase-producing bacteria [16-18]. Although routine procedures were in place in the hospital department, the strain quickly spread within one ward to another patient. After the death of the second patient and the transfer of the first patient to the long-term intensive care unit centre, the department was closed for two weeks and general cleaning including decontamination of all equipment was undertaken. No NDM-1-producing strain has been detected after the cleaning. Due to the importance of international travel in the spread of bacterial resistance, fast detection and active surveillance of bacteria producing acquired carbapenemases is needed [5-7,10,16,18,19].

We also tested the new MALDI-TOF mass spectrometry approach [3] for the detection of carbapenemase activity in the isolates. Although phenotypical detection of carbapenem-hydrolyzing enzymes in *A. baumannii* seems to be difficult by conventional methods [20], we were able to see a clear carbapenemase activity by this assay. Further validation, however, is necessary.

Table

Antimicrobial susceptibility of the NDM-1-producing *Acinetobacter baumannii* isolates, Czech Republic, July 2011 (n=2)

Antibiotic	MIC [µg/ml]
Ampicillin-sulbactam	32
Piperacillin	› 64
Piperacilin-tazobactam	› 64
Ceftazidime	› 32
Cefepime	› 32
Meropenem	› 32
Ciprofloxacin	› 32
Nalidixic acid	› 64
Gentamicin	32
Amikacin	32
Tetracycline	64
Chloramphenicol	8
Colistine	‹ 0.5
Trimethoprim-sulfamethoxazole	16

MIC: minimum inhibitory concentrations; NDM-1: New Delhi metallo-beta-lactamase-1.

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Microbiological and molecular characteristics of carbapenemase-producing *Klebsiella pneumoniae* endemic in a tertiary Greek hospital during 2004-2010

A Zagorianou[1], E Sianou[1], E Iosifidis[2], V Dimou[1], E Protonotariou[1], S Miyakis[3], E Roilides (roilides@med.auth.gr)[2], D Sofianou[1]
1. Department of Clinical Microbiology, Hippokration General Hospital, Thessaloniki, Greece
2. Infectious Diseases Unit, Third Department of Paediatrics, Aristotle University, Hippokration General Hospital, Thessaloniki, Greece
3. Third Department of Medicine, Aristotle University, Papageorgiou General Hospital, Thessaloniki, Greece

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We report 570 carbapenemase-producing *Klebsiella pneumoniae* (CPKP) clinical isolates in a 1,040-bed Greek tertiary hospital during 2004 to 2010. The first CPKP (VIM-producing) was isolated in September 2004. Despite initial containment, VIM producers have become endemic since 2006. KPC-producing *K. pneumoniae* was first isolated in August 2007 from a patient who came from Israel, spread rapidly, and outcompeted VIM. Overall, 267 (47%) VIM-producing and 301 (53%) KPC-producing strains were isolated, including 141 (24.7%) from patients with bacteraemia. Two isolates carrying both VIM and KPC were isolated in two consecutive months in 2009, but not since. The prevalence of CPKP increased from 0% in 2003 to 38.3% in 2010 ($p<0.0001$). All genotyped KPC producers harboured bla_{KPC-2} and belonged to two clones, among which the hyperepidemic Greek clone, related to those from the United States and Israel, predominated. Most metallo-beta-lactamase (MBL) producers carried the bla_{VIM-1} gene and belonged to several clones, whereas all but one isolate with bla_{VIM-12} were clustered within a five-month period, arising from one clone. Resistance to non-beta-lactam antibiotics was also increased among CPKP. They were almost invariably resistant to ciprofloxacin and trimethoprim-sulfamethoxazole. Resistance to colistin increased from 3.5% (4/115) in 2008 to 20.8% (25/120) in 2010, and resistance to tigecycline also increased. Following reinforcement of infection control measures, prevalence of CPKP (mainly KPC) has been reduced since mid-2009 (from 46% in 2009 to 38.3% in 2010). In view of the exhaustion of available therapies, investment in infection control resources and optimal antibiotic use is urgently required.

Introduction

Carbapenems are important therapeutic agents for treating infections caused by multi-drug resistant Gram-negative bacteria. Their efficacy, however, is threatened by the emergence of resistant isolates. In Greece (and elsewhere in Europe) a common mechanism is acquisition of hydrolytic enzymes (carbapenemases) inactivating beta-lactams [1,2]. The genes encoding carbapenemases are located on mobile genetic elements, allowing them to spread. Other mechanisms of resistance to carbapenems include the combination of extended spectrum beta-lactamase (ESBL) production with porin changes and/or upregulated efflux pumps (the latter particularly common among carbapenem-resistant *Pseudomonas aeruginosa*) [3,4].

Carbapenemases were initially found in non-fermenting bacteria; however, among *Enterobacteriaceae*, *Klebsiella pneumoniae* strains carrying acquired carbapenemases are increasingly reported [5]. The most prevalent carbapenemases are the molecular class B metallo-beta-lactamases (MBLs), mainly of VIM- and IMP-type, and the (class A) *K. pneumoniae*-carbapenemases (KPCs) [5]. More recently, outbreaks have been described of *K. pneumoniae* carrying the carbapenemases OXA-48 (Ambler class D) [6] and the New Delhi MBL (NDM-1) [7]. Carbapenemase-producing *K. pneumoniae* (CPKP) have been isolated worldwide, including most European countries [8]; CPKP are nowadays endemic in Greece (both VIM and KPC) and Israel (KPC). The presence of KPC in Israel was first reported in 2005 [9] and in Greece in 2007 [10].

Hippokration is a 1,040-bed, tertiary-care hospital in northern Greece, with all medical, surgical and paediatric subspecialties, a solid-organ transplantation unit and four intensive care units. The first CPKP in Hippokration Hospital was isolated in September 2004. Herein, we describe a seven-year study of the microbiological and molecular characteristics of *K. pneumoniae* producing different MBL- and KPC-type carbapenemases, endemic in this institution since 2006.

Methods

Between September 2004 and December 2010, we collected all *K. pneumoniae* isolates from clinical specimens (one per patient) that had a minimum inhibitory concentration (MIC) of >1 mg/L imipenem and stored them at -74 °C in 1% proteose-peptone containing 7% glycerol for further evaluation. Bacterial identification to species level and initial antibiotic susceptibility testing were performed with the VITEK2–automated system (bioMérieux, Marcy l'Etoile, France). Isolates were tested for tigecycline and colistin using the Etest (AB Biodisk, Solna, Sweden). For tigecycline, the breakpoints recommended by the United States Food and Drug Administration were used (susceptible: MIC ≤2 mg/L; resistant: MIC ≥8 mg/L). For colistin, the breakpoints recommended by the Clinical Laboratory Standards Institute (CLSI) for *Acinetobacter* spp. were used (susceptible: MIC ≤2 mg/L; resistant: MIC ≥4 mg/L) because there are no established CLSI MIC breakpoints against colistin for *Enterobacteriaceae*. All isolates were phenotypically screened for MBL- and KPC-type carbapenemases, using the imipenem/EDTA double-disk synergy test [11] and the imipenem/boronic acid combined-disc test [12], respectively.

Following phenotypic identification, MBL- and KPC-producing isolates were grouped according to their susceptibility profile (data not shown). However, no clear relationship between specific susceptibility profiles and clones could be identified with certainty. We selected 152 strains randomly (one of four) from each group, spanning all study years, for PCR amplification and sequencing, using primers specific for bla_{VIM}, bla_{IMP} and bla_{KPC}, as previously described [13,14]. The MICs of imipenem, meropenem and ertapenem for those 152 isolates were confirmed with the CLSI broth microdilution method [15], using *Escherichia coli* ATCC 25922 as control. The relatedness of isolates was determined by enterobacterial repetitive intergenic consensus (ERIC) PCR using the primer ERIC-2 [16].

Results

During the study period, 570 CPKP were isolated from clinical samples: blood (n=141; 24.7%), urine (n=166; 29.1%), surgical wounds (n=94; 16.5%), bronchial aspirates (n=35; 6.2%), central venous catheter tips (n=44; 7.7%), drainage sites (n=43; 7.5%), abscesses (n=17; 3.0%) and other sites (n=30; 5.3%, including cerebrospinal fluid, pleural or peritoneal tap, etc).

CPKP were isolated in all departments and 46.1% of isolates derived from two units: the eight-bed intensive care unit (ICU) (154 isolates; 27.0%) and the 10-bed organ transplant unit (109 isolates; 19.1%). The remaining CPKP were isolated in the hospital's surgical wards (146 isolates; 25.6%), the medical wards (139 isolates; 24.4%) and the paediatric/neonatal wards (22 isolates; 3.9%). The overall prevalence of CPKP among *K. pneumoniae* in the hospital increased from 0% in 2003 to 38.3% in 2010 (p<0.0001).

VIM-producing *Klebsiella pneumoniae*

In our hospital, the first CPKP was isolated in September 2004 from an infected wound of a patient who had been transferred from the ICU to the orthopedic ward; The MIC of imipenem and meropenem were 4 and 2 mg/L, respectively. Phenotypic testing revealed synergy between imipenem and EDTA, and the presence of the bla_{VIM-1} gene was identified. A further seven VIM-1 producing CPKP were isolated in the following three months: four in the ICU, two in surgical ward and one in the transplantation unit. Following rigorous infection control measures, the outbreak temporarily ceased, and only five sporadic cases occurred over the following 14 months. A new wave started in March 2006; since then CPKP have been endemic in the hospital. The outbreak trend is depicted in Figure 1.

KPC-producing *Klebsiella pneumoniae*

In August 2007, a *K. pneumoniae* isolate resistant to imipenem (MIC>16 mg/L) was recovered from a central venous catheter tip of a Dutch tourist, who was admitted to the ICU of our hospital after a stay in Israel. Unlike the previous CPKP isolates, the isolate from this patient was negative in the imipenem-EDTA test. This strain was resistant to aztreonam, and synergy was demonstrated between amoxicillin/clavulanic acid and cefotaxime. A positive imipenem/boronic acid test suggested the presence of KPC, which was confirmed by bla_{KPC} sequencing. KPC-producing organisms were initially confined to the ICU and organ transplant unit. In October 2007, they were isolated in surgical wards (orthopedic) and later in the same month in medical wards (renal, neurology).

KPC spread rapidly in the hospital, becoming increasingly prevalent (from 21 isolates in 2007 to 134 in 2009), while VIM-producing isolates declined (from 77 isolates in 2007 to 25 in 2009) (Table 1).

Since mid-2009, the prevalence of KPC isolates has been gradually declining, whereas the much lower rate of VIM producers has slightly increased (Figure 2). In May and June 2009, two (to date unique) isolates were identified that carried both bla_{KPC} and bla_{VIM} [17]. Table 2 summarises the CPKP isolated in the different wards/departments of the hospital over the study period.

Phenotypic and genotypic analysis of isolates

Overall, 267 (47%) isolates were phenotypically characterised as MBL- and 301 (53%) as KPC-producing (Table 1). All 70 genotypically tested KPC producers harboured the bla_{KPC-2} gene. Among MBL producers, molecular analysis revealed the presence of 72 bla_{VIM-1} and 10 bla_{VIM-12} genes. The latter were clustered between November 2006 and April 2007, with the exception of one sporadic case in June 2007. ERIC analysis revealed several different patterns among VIM producers, including a distinct clone comprising all VIM-12 carrying strains. KPC producers belonged to two different clones, one being predominant (data not shown).

Resistance to imipenem and meropenem, as well as of MBL producers to ertapenem, was variable (MIC ranging from 2 to >32 mg/L). KPC producers had invariably a MIC of >32 mg/L to ertapenem. Notably, 87 (32.6%) VIM-producing isolates were resistant to aztreonam (an antibiotic stable to the hydrolytic activity of MBLs). PCR analysis revealed that these 87 isolates also contained an extended-spectrum beta-lactamase (ESBL) gene, bla_{SHV-12} or bla_{SHV-5}.

Antimicrobial susceptibilities to non-beta-lactam agents are reported in Table 3. Almost all CPKP isolates were resistant to ciprofloxacin and trimethoprim-sulfamethoxazole. Gentamicin was more active than amikacin in vitro, particularly among KPC producers. Of note, 18.6% (56/301) of KPC-producing isolates were also resistant to colistin. Among CPKP, colistin resistance increased from 3.5% (4/115) in 2008 to 20.8% (25/120) in 2010. Tigecycline resistance, although less frequent than colistin, also increased among KPC producers (Table 3).

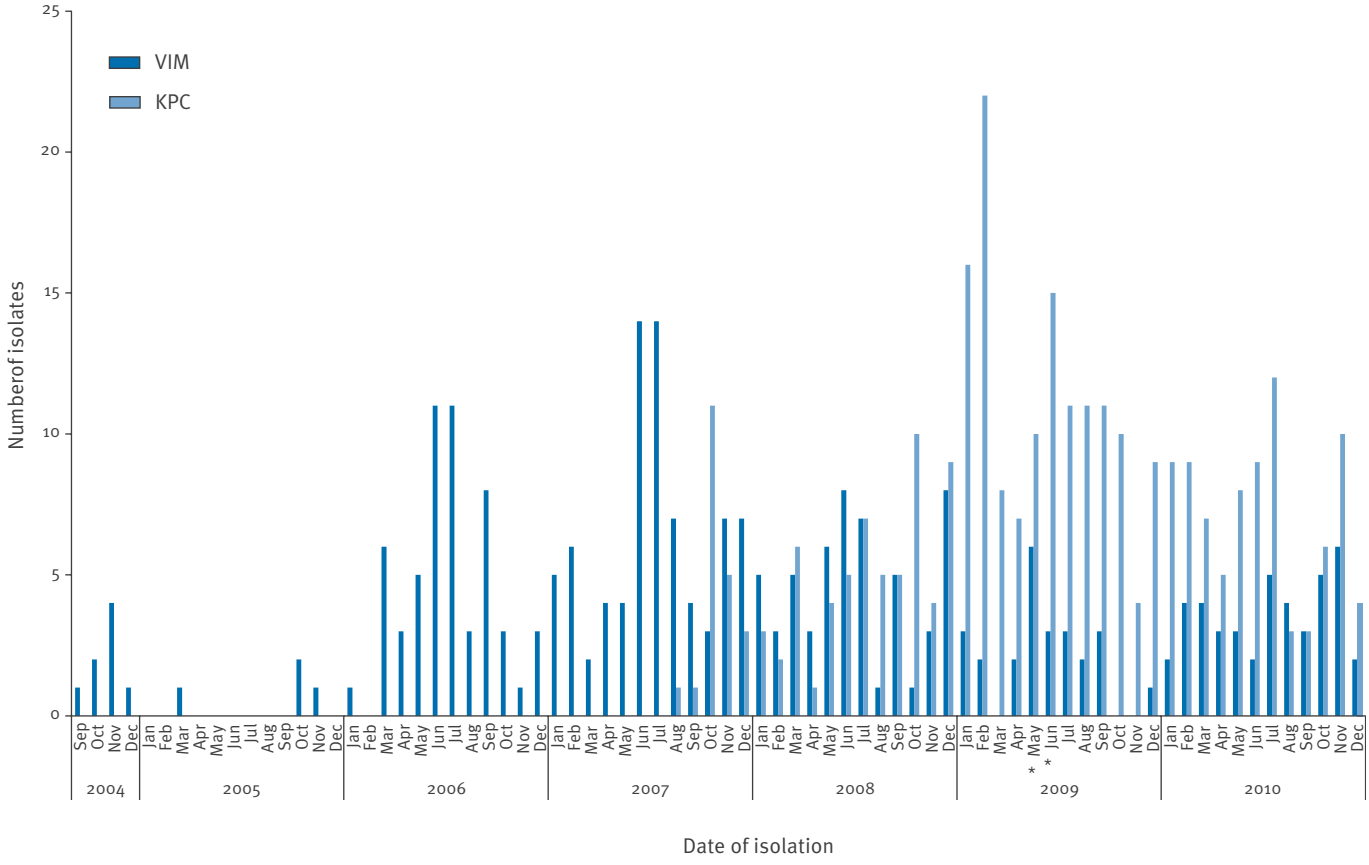
Discussion and conclusion

This study describes the, to our knowledge, largest outbreak of CPKP in a healthcare institution. As only clinical specimens from unique patients were included, and the MIC threshold for carbapenemase testing (imipenem >1 mg/L) was higher than the subsequently defined epidemiological cut-off values [18], it is possible that the prevalence of CPKP was underestimated. We observed on the one hand the dynamic co-existence of VIM- and KPC-producing strains, where KPC producers outcompeted pre-existing VIM producers, and on the other hand the rare emergence of strains co-producing VIM and KPC appearing late in this epidemic.

ERIC results suggest both clonal expansion and horizontal transmission of resistance determinants. Although all early VIM-1 producing isolates belonged to the same clone [14], subsequent VIM producers belonged to multiple distinct clones; multi-clonality of VIM-producing CPKP circulating in Greek hospitals was supported by previous reports [19]. All KPC producers belonged to two clones, the predominant of which likely corresponded to the hyperepidemic Greek clone, related to those from the United States and Israel; this has also been shown previously for some of our samples, using pulsed-field gel electrophoresis (PFGE) [13]. Notably, our index KPC strain was isolated from a patient who had been to Israel. To the best of

Figure 1

Clinical isolates of *Klebsiella pneumoniae* producing VIM or KPC, in Hippokration hospital, Thessaloniki, Greece, September 2004–December 2010 (n=570)



KPC: *Klebsiella pneumoniae* carbapenemase; VIM: Verona integron-encoded metallo-beta-lactamase.

Note: Two strains that produced both VIM and KPC are not included in the graph itself. However, their isolation dates (May and June 2009) are indicated by asterisks.

our knowledge, this is the first report of a documented transfer of KPC from Israel to Greece. Remarkably, the timing of this transfer coincided with the peak of the KPC outbreak in Israel [20]. These data are in accordance with previous findings on the similarities of KPC-producing clones reported from those two countries in 2007 [13]. It should be noted that KPC-2 strains had been reported from another area in Greece (Crete) earlier in the same year [21], but the origin of those isolates was unknown.

Standard infection control measures (including contact precautions) were implemented at the beginning of the outbreak in Hippokration hospital. However, adherence to the measures seemed to subside after the first months and CPKP were transmitted more widely. The highest incidence of CPKP was monitored in 2009, and since then infection control policy has been reassessed and intensified by the infection control committee (infectious disease physicians, the head of the microbiology department, infection control nurses and physicians from other hospital departments) according to guidelines from the Centers for Disease Control and Prevention [22].

The number of hospital infection control nurses was increased from one to three, and they actively monitored adherence and effectiveness of intensified interventions under the guidance of an infectious disease physician. Staffing levels in all hospital departments, including the ICUs, were reassessed. Moreover, the accurate identification of CPKP was verified and the presence of carbapenemase-producing bacteria was communicated in written reports to all physicians. Affected areas received quality visits from infection

Table 1

Klebsiella pneumoniae isolates carrying VIM and/or KPC in Hippokration hospital, Thessaloniki, Greece, September 2004–December 2010 (n=570)

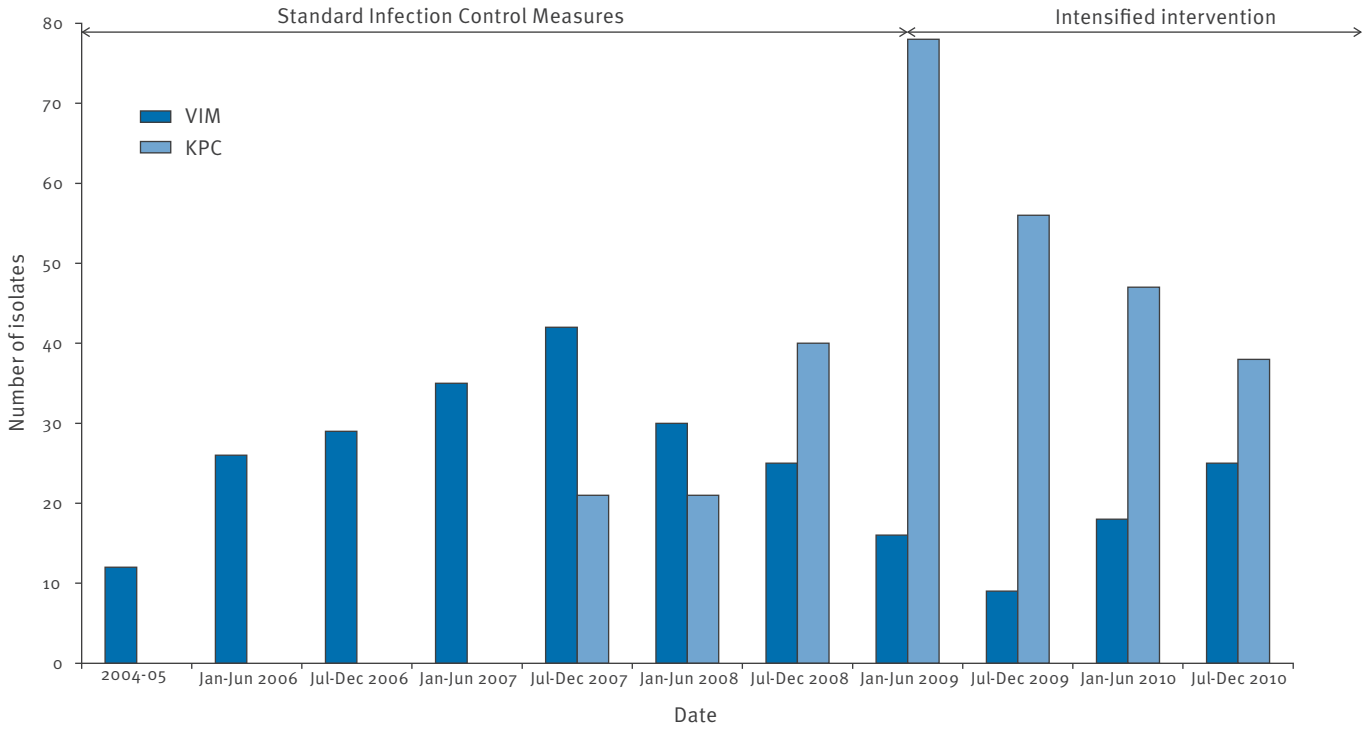
Year	All CPKP	VIM (% of all CPKP that year)	KPC (% of all CPKP that year)
2004	8	8	0
2005	4	4	0
2006	55	55	0
2007	98	77 (78.6)	21 (21.4)
2008	116	55 (47.4)	61 (52.6)
2009	161[a]	25 (15.7)	134 (84.3)
2010	128	43 (33.6)	85 (66.4)
Total	**570[a]**	**267 (47.0)**	**301 (53.0)**

CPKP: Carbapenemase-producing *Klebsiella pneumoniae*; KPC: *Klebsiella pneumoniae* carbapenemase; VIM: Verona integron-encoded metallo-beta-lactamase.

[a] Two strains producing both VIM and KPC were isolated in 2009 and are not included in the columns of VIM- and KPC-expressing isolates.

Figure 2

VIM- and KPC-producing *Klebsiella pneumoniae* isolates, Hippokration hospital, Thessaloniki, Greece, September 2004–December 2010 (n=568)



KPC: *Klebsiella pneumoniae* carbapenemase; VIM: Verona integron-encoded metallo-beta-lactamase.
Data shown by half year, except for the period 2004-2005, when VIM producers occurred only sporadically.

control nurses. Feedback from those visits,as well as resistance rates were communicated to the infectious disease physicians. Due to the endemic situation only infections with CPKP were monitored and no active surveillance took place. A database of all patients with CPKP was generated and distributed to the microbiology department, infection control nurses and infectious disease physicians. Incidence rates of CPKP were reported weekly to all hospital departments as well as the hospital's administration. Patients' location and transfer between departments and/or hospitals were monitored daily. Infection control precautions to prevent patient-to-patient transmission were intensified and targeted patients with CPKP. Contact precautions were put in place for all patients with a positive CPKP test: Where feasible, a single patient room was used for isolation. However, cohorting of patients was also used in departments where single patient rooms were not available (including all ICUs). Environmental measures were also implemented including dedicated use of non-critical equipment. Surface cleaning and disinfection was reinforced as well as final cleaning after a patient was moved from a department.

In February 2009, the infection control committee and hospital administration decided not to accept new admissions to adult ICU for 10 days. Education of healthcare personnel was intensified using audits, posters and video presentations about hand hygiene, contact precautions and severity of infections due to CPKP. Judicious use of antimicrobials was encouraged and daily quality rounds and audits of antimicrobial prescriptions were implemented. An antimicrobial restriction policy was in place and all antimicrobials with extended spectrum (especially carbapenems) were closely monitored by an infectious disease physician in cooperation with the hospital's pharmacy. Given the magnitude of the problem in Greece, 'Procrustes', a nationwide action plan for the containment of carbapenem-resistant bacteria has been implemented as of November 2010; its main features have been outlined elsewhere [2]. With these measures in place, a reduction in the prevalence of CPKP in the hospital was recorded (from 46% of *K. pneumoniae* strains isolated in 2009 to 38.3% in 2010, see Figures 1 and 2).

Not surprisingly [1], this outbreak has as yet not been contained, despite hospital-wide reinforcement of infection control measures. It is likely that its appearance and perpetuation had multiple contributors. Those included breaches in infection control practice, like low compliance with hand hygiene [23] and contact precautions. Inter-hospital transfer of carriers is favoured in Greek hospitals because there is no integrated recording system of re-admission alerts and inter-hospital communication [1]. Antibiotic overuse is an important contributor for the emergence and spread of resistance; association between carbapenem consumption and resistance has been previously documented [24]. As per institutional policy, in departments with a

Table 2

Carbapenemase-producing *Klebsiella pneumoniae* by unit/ward, Hippokration hospital, Thessaloniki, Greece, September 2004–December 2010 (n=570)

Year	Intensive care unit (%)	Transplant unit (%)	Medical wards (%)	Surgical wards (%)	Paediatric wards (%)	**Total**
2004	4	1	0	3	0	**8**
2005	1	1	1	1	0	**4**
2006	27 (49.1)	7 (12.7)	9 (16.4)	10 (18.2)	2 (3.6)	**55**
2007	25 (25.5)	23 (23.5)	21 (21.4)	26 (26.5)	3 (3.1)	**98**
2008	35 (30.2)	23 (19.8)	30 (25.9)	23 (19.8)	5 (4.3)	**116**
2009	39 (24.2)	28 (17.4)	42 (26.1)	46 (28.6)	6 (3.7)	**161**
2010	23 (18.0)	26 (20.3)	36 (28.1)	37 (28.9)	6 (4.7)	**128**
Total	**154 (27.0)**	**109 (19.1)**	**139 (24.4)**	**146 (25.6)**	**22 (3.9)**	**570**

Table 3

Susceptibility profile of carbapenem-resistant *Klebsiella pneumoniae* isolates to non-beta-lactam antimicrobial agents in Hippokration hospital, Thessaloniki, Greece, September 2004–December 2010 (n=568)

K. pneumoniae (no of isolates)	Number (%) resistant					
	GEN	AMK	CST	TGC	CIP	SXT
VIM producers (n=267)	63 (23.6)	79 (29.6)	9 (3.4)	14 (5.2)	257 (96.3)	264 (98.9)
KPC producers (n=301)	44 (14.6)	223 (74.0)	56 (18.6)	34 (11.3)	295 (98.0)	274 (91.0)

AMK: amikacin; CIP: ciprofloxacin; CST: colistin; GEN: gentamicin; KPC: *Klebsiella pneumoniae* carbapenemas; SXT: trimethoprim-sulfamethoxazole; TGC: tigecycline; VIM: Verona integron-encoded metallo-beta-lactamase.

high prevalence of CPKP, i.e. the ICU, colistin and gentamicin are used as initial empirical treatment when an infection with such an organism is suspected [25].

Notably, we observed different resistant profiles within clones in this study. Reasons for this may include the presence of additional mechanisms contributing to resistance patterns (typically, the frequent co-existence of an ESBL-type enzyme and VIM), the concurrent existence of several clones of VIM-producing strains, but also the increased rates of non-susceptibility to tigecycline and/or colistin, probably as a result of increasing use of those antibiotics for the treatment of infections with carbapenem-resistant organisms. Of particular concern are our results showing frequent aztreonam resistance among VIM producers, due to the additional carriage of an ESBL, as well as the high rates of resistance to non-beta-lactam agents, particularly among KPC producers. In agreement with recent reports [26, 27], increasing colistin resistance underlines a real threat from the emergence of multi- or pandrug-resistant bacteria. In view of the exhaustion of available therapeutic options, investment in infection control resources and optimal antibiotic use, along with co-ordinated efforts from all involved parties is urgently required.

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Emergence and outbreak of carbapenemase-producing KPC-3 *Klebsiella pneumoniae* in Spain, September 2009 to February 2010: control measures

A Robustillo Rodela[1], C Díaz-Agero Pérez[1], T Sanchez Sagrado[1], P Ruiz-Garbajosa[2], M J Pita López[1], V Monge (vmonge.hrc@salud.madrid.org)[1]

1. Service of Preventive Medicine, University Hospital Ramón y Cajal, and Health Research Institute (Instituto Ramón y Cajal de Investigación Sanitaria; IRYCIS), Madrid, Spain
2. Microbiology Service, University Hospital Ramón y Cajal, Biomedical Research Centre Network for Epidemiology and Public Health (CIBER en Epidemiología y Salud Pública) and Health Research Institute (Instituto Ramón y Cajal de Investigación Sanitaria; IRYCIS), Madrid, Spain

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This report describes the epidemiological features of the first outbreak caused by KPC3 carbapenemase-producing *Klebsiella pneumoniae* (KPC-3-KP) in Spain and how it was effectively controlled. From 16 September 2009 to the end of February 2010, seven patients infected or colonised with KPC-3-KP were detected. Stool surveillance cultures were recovered from patients, doctors, nurses, nursing assistants, cleaners and hospital porters working in the affected units. Hand swabs were taken from workers and patients' relatives for culturing. Environmental samples were also taken. Patients infected or colonised with KPC-3-KP were placed in single rooms under contact precautions and 4% chlorhexidine soap was used for their daily hygiene. Staff attended educational seminars and workshops on hand hygiene and isolation of patients. An alcohol-based disinfectant was used for surface cleaning and disinfecting. The floor was cleaned with a disinfectant containing benzalkonium chloride and didecyldimethylammonium. All samples collected were negative for KPC-3-KP. After implementing the control measures, no further cases were reported in the affected units. All cases had comorbidities, long hospital stay and aggressive/intensive antimicrobial treatment. This study emphasises the importance of early intensification of infection control to interrupt the transmission of KPC-producing organisms.

Introduction

Carbapenems are widely regarded as the drugs of choice for the treatment of severe infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* [1]. The emergence of carbapenem-resistant enterobacteria is worrisome, since antimicrobial treatment options are very restricted [2].

Carbapenemases are a large and diverse family of microbial enzymes that hydrolyse not only carbapenems but also other beta-lactam antibiotics. One of the most important groups is the KPC-enzymes, classified as beta-lactamases Ambler class A and Bush functional group 2, that hydrolyse all beta-lactams except cephamycins [3].

KPC-producing *Klebsiella pneumoniae* were first isolated in North Carolina in 1996 [4] and until 2004 these enzymes were found only in the United States [5-7]. The first outbreak of KPC-producing *K. pneumoniae* outside the United States was described in Tel Aviv in 2006 [8]. The strains isolated in Israel were genetically identical to the ones previously isolated in the United States. This supported the hypothesis that the KPC producer was transferred from the United States to Israel [9]. The European country in which most cases have been reported so far is Greece, where the situation can be described as endemic [10-15]. Italy and France have recently described a rapid increase in the number of of cases [16,17].

We report here the epidemiological features of the first outbreak by KPC3 carbapenemase-producing *K. pneumoniae* (KPC-3-KP) in Spain and how it was controlled.

Material and methods

Outbreak investigation

Ramon y Cajal Hospital is a 1,090-bed university teaching hospital located in Madrid, Spain. From 16 September 2009 through the end of February 2010, seven patients with infection or colonisation with KPC-3-KP were detected. Four of these patients were admitted to two different but adjacent units: internal medicine and oncology. Both units share hospital porters and cleaning staff and some medical equipment

such as electrocardiographs. Of the remaining three patients, one was detected in the paediatric unit on a different floor during the same period, and the other two appeared after hospital discharge.

Case patient definition

Any inpatient infected or colonised with KPC-3-KP was considered as a case. An infected patient was defined as a person with a positive culture for KPC-3-KP who met the Centres for Disease Control and Prevention (CDC) clinical criteria of infection [18]. A patient was defined as colonised, when KPC-3-KP was isolated from surveillance cultures or clinical specimens in the absence of clinical signs of infection.

Clinical data, surveillance, and environmental cultures

Clinical records from inpatients were reviewed and the following data were registered: age, sex, diagnosis at the time of hospital admission, comorbid conditions (diabetes mellitus, cardiovascular disease, pulmonary disease, hepatic disease, central nervous system disease, malignancy, anaemia), previous hospital admissions, admission to intensive care unit, treatment with immunosuppressors, antibiotic treatment, invasive procedures (insertion of central venous catheter, insertion of urinary catheter, surgery, mechanical ventilation), antimicrobial resistance pattern, and outcome (recovery/death).

According to infection control and prevention policy in our institution, when a patient has present or previous history of infection/colonisation with a multidrug-resistant microorganism or has shared a room with an infected/colonised patient, rectal and pharyngeal swabs are taken to detect colonisation and to decide when to discontinue contact precautions.

During the outbreak, stool surveillance cultures were recovered from patients, doctors, nurses, nursing assistants, cleaners and hospital porters working in the internal medicine and oncology units [19]. Hand surface swabs were taken from workers and patients' relatives and cultured. Subjects placed their fingertips on cystine lactose electrolyte deficient (CLED) agar plates using the four fingers first, followed by the thumb in the middle of the plate. An imprint method was used because it is easy and feasible in our institution. Moreover, with this method we estimated bacteria present on the palm of the hand, which is the anatomical location with the highest risk of transmission. Samples were also taken from working surfaces, taps, patients' rooms (bedrails, sinks, taps and bedside tables), nurses' sinks, computer key boards, pulse oximeters, and sphygmomanometers. Stool and environmental samples were directly inoculated onto MacConkey agar plates supplemented with ceftazidime (4 mg/L) and using previous broth enrichment (BHI) supplemented with imipenem (1 mg/L).

Bacterial identification, susceptibility testing, screening for carbapenemase production and molecular laboratory techniques were done as described by Curiao et al. [20].

Outbreak control measures

Patients were placed in single rooms under contact precautions according to our facility's protocol. A 4% chlorhexidine soap was used for their daily hygiene. Staff working in the affected units received educational seminars and attended workshops on special hand hygiene and patient isolation. The units were cleaned thoroughly with two products that were also used for the daily cleaning during the whole outbreak period. An alcohol-based disinfectant was used for surface cleaning and disinfecting (Incidin Liquid), and the floor was cleaned with a disinfectant containing benzalkonium chloride and didecyldimethylammonium (Incidin Rapid).

Results

The outbreak described here involved seven patients. The index case (Case 1) was a patient in their 60s who had no history of previous hospitalisations. Three months after admission to the oncology unit (third floor, section A), KPC-3-KP was isolated in a urine culture. The patient was discharged five days later.

Two further cases were detected in the adjacent internal medicine unit (third floor, section B) 12 and 37 days after isolation of Case 1 (see Figure). Neither case had a history of previous admission to our hospital. Case 2, a patient in their 50s, had a history of rectal colonisation with ESBL-producing *Escherichia coli*. Ten days after admission KPC-3-KP was isolated in a rectal culture during a routine investigation of rectal carriage of ESBL-producing isolates. Case 3 had symptoms of urinary tract infection and the urine culture was positive for KPC-3-KP. ESBL-producing *Klebsiella oxytoca* and *E. coli* were isolated in rectal swabs as well. This patient shared the same room with Case 2 for four days one month before KPC-3-KP isolation.

Case 4 was a teenager admitted to the paediatric unit, (located on the tenth floor). KPC-3-KP was isolated in a wound culture. This patient had previous hospitalisations in our institution, the last one a two-month stay from which they had been discharged only ten days before. This patient had never been admitted to the oncology or the internal medicine unit, and the paediatric unit does not share staff or medical equipment with those units.

Two months after the first case, KPC-3-KP was isolated in a rectal culture from a patient in their 60s (Case 5) who was staying for one month in the oncology unit, at the same time as Case 1, but they did not share the same room at any time. At the time screening for KPC-3-KP was performed in the hospital, Case 5 had already been discharged. It was during his next admission, two months after Case 1, that control surveillance cultures

were grown and KPC-3-KP was isolated in a rectal culture. No further cases have been detected since, neither in the oncology unit nor in the internal medicine unit.

Two new cases were reported two months after Case 5. From Case 6 KPC-3KP was isolated in blood and urine cultures taken in the emergency unit. This case was a person in their late 80s and had been an inpatient for 10 days during the outbreak period but had not stayed in the affected units. The last case detected (Case 7) a person in their 70s previous history of multiple hospitalisations in our institution, was admitted on January 2010. During her stay KPC-3-KP was isolated in a sputum culture. She had been referred to the gastroenterology unit during the outbreak period and she had no known epidemiological association with the rest of the cases. The sequence of case detection is shown in the Figure.

All patients were diagnosed between 10 and 61 days after admission to hospital. All of them had multiple underlying conditions (anaemia: n=5; hypertension n=4; cancer: n=3; diabetes mellitus: n=1; Crohn disease: n=1; chronic renal insufficiency: n=1). Five of them were on immunosuppressive treatment. All cases received antibiotic therapy in the month prior the diagnosis: three with amoxicilin/clavulanic , three with meropenem, two with teicoplanin and one with vancomycin.

Microbiological results

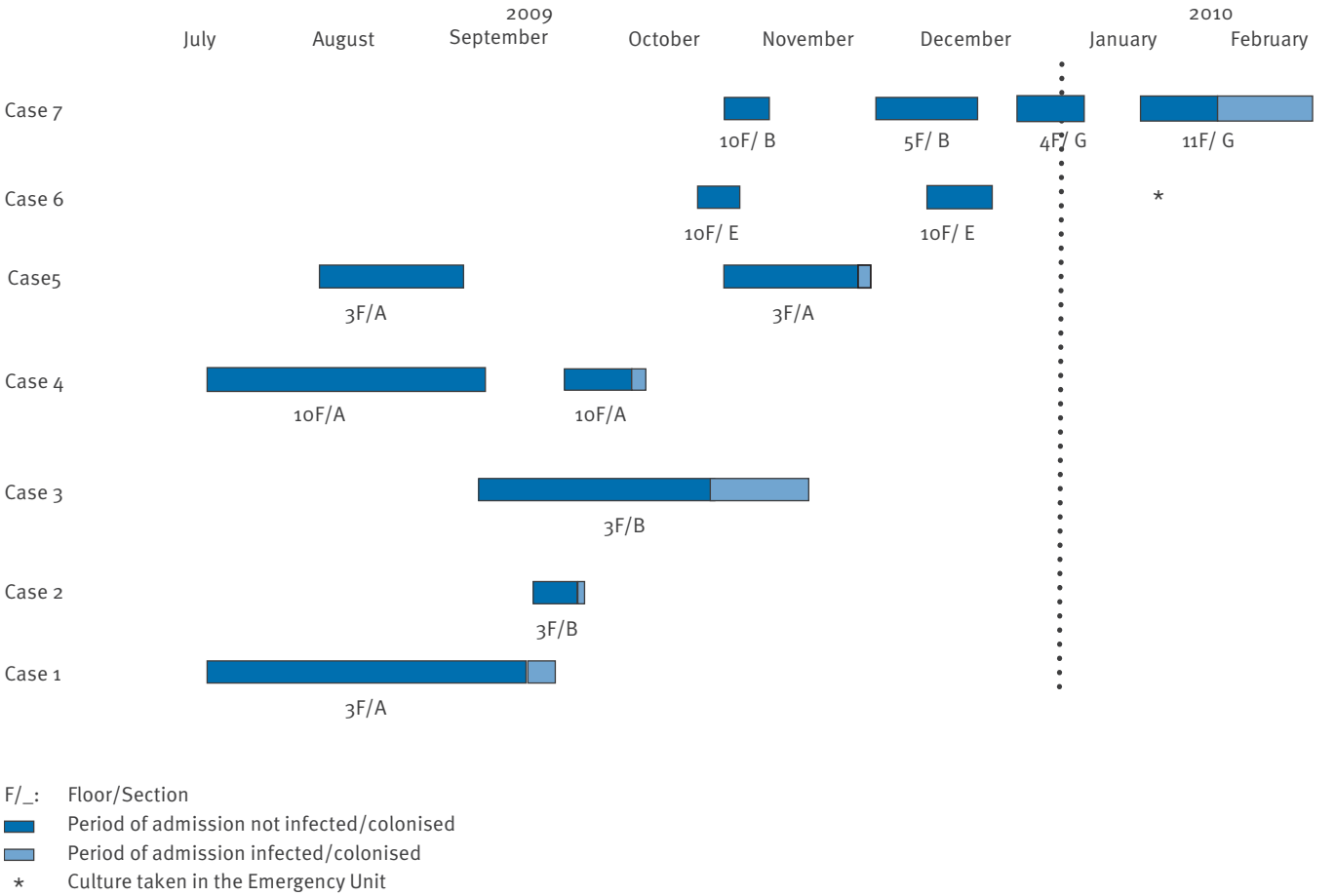
All these isolates were carbapenemase-producing KPC 3. They were considered clonally related and were assigned to the new sequence type ST384. All the isolates were susceptible to amikacin, gentamicin, ciprofloxacin and trimethroprim/sulfamethoxazole and resistant to imipenem, meropenem, cefepime, cefotaxime and piperacillin/tazobactam.

Stool samples were collected from 25 of the other 32 inpatients in the oncology and internal medicine units and from 27 of 39 staff members. All of them were negative for KPC-3-KP.

The 13 hand cultures from health staff were also negative for KPC-3-KP, and the microorganisms isolated were all common skin contaminants. ESBL-producing *K. pneumoniae* was isolated in only one healthcare worker. All five hand samples taken from the patients'

Figure

Admission period and hospital unit of patients with KPC3-producing *Klebsiella pneumoniae*, Madrid, Spain, 16 September 2009–February 2010 (n=7)



F/_: Floor/Section
Period of admission not infected/colonised
Period of admission infected/colonised
* Culture taken in the Emergency Unit

relatives were negative for KPC-3-KP. The 13 environmental samples were also negative for KPC-3-KP, but positive for other microorganisms as listed in the Table.

Discussion

We describe the first cases of infection/colonisation with KPC-3 carbapenemase-producing *K. pneumoniae* in Spain. Microbiological data, PCR analysis and molecular techniques showed that all isolates were genetically identical, supporting the hypothesis for a clonal KPC-3-related infectious outbreak [20]. Emergence of KPC-producing *K. pneumoniae* is a significant public health concern.

Outbreaks have been reported in several countries [7,8,13,21-23]. In some of those outbreaks, the index case had been previously admitted to a hospital in an endemic area [22,23]. In our study, none of the patients had a previous history of travelling to endemic areas. KPC-3-KP may have been introduced to our facility by an undetected infected or colonised patient, or the index case may have been previously colonised before the KPC-3-KP isolation.

Infection control measures were intensified on the oncology, internal medicine and paediatric wards, where the first five cases were detected. Cases 6 and 7 had been admitted to the hospital during the outbreak period in October 2009, but not to any of the affected units, so no samples had been taken. They were identified as colonised/infected by the same clone only during a later hospital admission in early 2010. Other authors have reported outbreaks in which associated cases were detected from two weeks to five months after the end of the defined outbreak period [21]. We do not know if Cases 6 and 7 were colonised during their earlier hospitalisation in October 2009, since no screening was performed at the time, or if they may have been colonised before admission to the hospital (January–February 2010), since the prevalence of KPC-producing *K. pneumoniae* in the community is unknown.

Studies from Israel and the United States have identified risks factors for nosocomial acquisition of KPC-producing *K. pneumoniae*. They included poor functional status, ICU stay, transplantation, mechanical ventilation, prolonged hospital stay and antimicrobial treatment [24-26]. Another study that took place in Puerto Rico [21] described further risk factors: wounds, previous surgery and transfer between hospital units. We cannot extract conclusions related to risks factors for KPC-3-K from our study. All the cases detected in our facility had similar characteristics to the ones affected in prior published outbreaks: comorbidities, long hospital stay [7,13,23] and aggressive/intensive antimicrobial treatments [5,13,15,21]. In our case, two patients were in an ICU with mechanical ventilation and four underwent surgical procedures.

Antimicrobial pressure may have been a selective factor for the primary colonisation. Use of extended spectrum carbapenems, fluorquinolones and cephalosporins has been identified as a risk factor for carbapenem-resistant *K. pneumoniae* [24-26]. None of our patients had received quinolones one month prior to the infection and all patients had received beta-lactams, as did the patients of the outbreaks described by Purnaras et al. [14] and by Nadkarn [27]. Further studies are needed to clarify the role of different antimicrobials in carbapenem-resistance acquired by *K. pneumoniae*. It may be plausible that the number of antibiotics administered to case patients increases the risk of acquisition of carbapenem-resistance in *K. pneumoniae*, rather than the administration of a specific antibiotic group [21].

Mortality data described in different papers range from 25% to 69% [7,13-15,21,26]. In our case series one death was reported. The only patient with bacteraemia by KPC-3-KP died shortly after hospital discharge, and the cause of death is unknown to us. The rest of the patients were successfully treated according to the susceptibility pattern.

Table

Environmental cultures, KPC3-producing *Klebsiella pneumoniae* hospital outbreak, Madrid, Spain, 16 September 2009–February 2010 (n=13)

Microorganism[a]	Surface
Staphylococcus epidermidis (n=8)	Bedside tables, bed rails, sinks, sphygmomanometer, working surfaces, computer keyboard
Non-fermenting Gram-negative bacilli (n=1)	Working surface
Streptococcus sp. (n=1)	Working surface
ESBL-producing *K. pneumoniae* (n=1)	Bedside table
K. pneumoniae (n=2)	Working surfaces, computer keyboard
Bacillus sp. (n=1)	Computer keyboard
Pseudomonas aeruginosa (n=2)	Taps
Enterobacter sp. (n=1)	Working surfaces

KPC-3-KP: KPC3 carbapenemase-producing *K. pneumoniae*

[a] >1 microorganism can be isolated in the same sample.

K. pneumoniae is a common cause of nosocomial pneumonia. Its principal nosocomial reservoirs are contaminated medical equipment, hands of hospital staff and the gastrointestinal tract of patients [9]. In our study, the emergence of a monoclonal outbreak and the impossibility of identifying a common source in an environmental reservoir suggest transmission from patient to patient through the hands of hospital staff, as has been previously described [23,28,29].

Some papers have reported the isolation of KPC-producing *K. pneumoniae* from surfaces, intravenous poles, blood pressure cuffs or endotracheal tube connectors [5,15], while other authors, as well as our own outbreak investigation, did not isolate KPC-3-KP in environmental samples [13,30]. However, after thorough cleaning and disinfection, the outbreak did not spread further. We may not have taken enough environmental samples.

Previous studies have shown that KPC-producing *K. pneumoniae* outbreaks are difficult to manage. Monoclonal outbreaks may evolve to polyclonal endemicity if a nosocomial pathogen is not controlled soon after its emergence in a hospital [14,21]. Our infection control measures, including contact isolation, were enhanced the moment the first case was detected, and they controlled the outbreak effectively. It is difficult to evaluate the effectiveness of each control measure (chlorhexidine soap, contact isolation, staff training, surface cleaning and disinfection with new products) since all of them were established immediately. The rapid simultaneous application of several measures may have contributed to the effectiveness. We assume that early communication to the staff of the affected units, educational talks and hand hygiene were key factors in controlling the outbreak.

The dissemination of carbapenem-resistant *K. pneumoniae* and the difficulty to treat infections produced by these bacteria with the currently available antimicrobial drugs make it necessary to focus our efforts on early detection and implementation of infection control measures to limit the emergence and transmission of KPC-producing organisms. This issue will require further investigation directed at characterising the molecular mechanisms and selection pressures which promote the spread of these microorganisms. Judicious antimicrobial use should be emphasised.

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Experiences from the Shiga toxin-producing *Escherichia coli* O104:H4 outbreak in Germany and research needs in the field, Berlin, 28–29 November 2011

STEC Workshop Reporting Group[1]
1. Members of the group and the corresponding author are listed at the end of the article

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This report presents the main findings from an international workshop on Shiga toxin-producing *Escherichia coli* (STEC), held on 28–29 November 2011, organised by the Robert Koch Institute [1]. The workshop assembled over 100 experts in clinical medicine, epidemiology, public health, microbiology, food safety, and environmental science from various countries.

The 2011 outbreak

From May to July 2011, Germany experienced an unprecedented outbreak of bloody diarrhoea and haemolytic uraemic syndrome (HUS) mostly among previously healthy adults caused by STEC serotype O104:H4 [2-4]. Travellers from several countries were affected and an autochthonous outbreak caused by the same strain occurred in France [5]. Fenugreek sprouts were identified as the vehicle of infection [6].

Although the outbreak was controlled successfully, significant scientific challenges remain. To address these challenges, the workshop organised four working groups to discuss research needs in the following areas: (i) clinical issues, (ii) epidemiology, prevention and burden of disease, (iii) microbiology and pathogenesis and (iv) food safety, zoonotic and environmental aspects. The major research needs are presented below.

Clinical issues

As an important characteristic of the outbreak, many patients experienced severe clinical symptoms, and case fatality was substantial. Almost 50% of the HUS patients needed dialysis and a similar proportion developed neurologic symptoms [7]. Meanwhile, the vast majority of patients have completely recovered. No causal therapy for HUS exists, and pooled analysis of previous data showed no benefit of antibiotic treatment. In this outbreak, novel strategies for treating patients with HUS were applied (eg, antibody treatment with Eculizumab, immunoadsorption). The overall goal should be to optimise early diagnosis and care for patients with STEC-associated disease to reduce mortality and prevent organ damage.

Disease course and outcome: manifestations and predictors

The natural disease course including the overall frequency and severity of the involvement of different organ systems should be investigated. Another aim is to study the risk factors and protective factors for the development of the different stages of STEC disease such as. bloody diarrhoea, HUS, neurological symptoms, recovery, and fatal outcome. This should include demographic and clinical characteristics, as well as genetic and environmental factors. Appropriate biomarkers (e.g. complement activation, chemokines, cytokines) indicating the risk of severe disease should be determined. Risk scores should be developed to identify and adequately treat patients at increased risk of HUS. The available clinical data could be used to develop an algorithm for standardised diagnostic procedures. The pathogenetic mechanisms underlying severe organ-specific lesions and recovery should be investigated (e.g. kidney, central nervous system).

Effectiveness and safety of treatment strategies

This outbreak provides a unique opportunity to study the effects of antibiotics on the disease course. The effectiveness and safety of antibody treatment and immunoadsorption (short and long term outcomes) should be studied. Moreover, new therapeutic strategies to effectively remove Shiga toxin from the gastrointestinal tract once STEC has been diagnosed should be developed. Based on the scientific data obtained in this outbreak, recommendations for the future use of new and already existing therapeutic measures and international study protocols for treatment of STEC-HUS are warranted.

Epidemiology, prevention and burden of disease

Research questions were collected and categorised according to high, moderate or low priority. Some areas may not belong to epidemiologic research in a stricter sense, but relate primarily to improving outbreak detection and control and STEC surveillance. The four topics outlined in the sections below were considered of utmost importance.

Laboratory surveillance for outbreak detection

Research should be targeted towards developing an optimal strategy for STEC surveillance. Diagnosis of all STEC is complex, but it is paramount to have a scheme allowing for timely assessment of the virulence profile of the causative agents and other characteristics (e.g. serotype) facilitating basic identification of clusters or of strains amenable for further subtyping (e.g. O157). The usefulness of improved microbiological methods for primary detection of STEC and other diarrhoeagenic *E. coli* for clinical and public health purposes should be studied. Molecular subtyping is a powerful tool for detecting and investigating food-borne outbreaks, and its implementation is recommended. Research should identify the most useful typing methods and evaluate its cost-effectiveness.

Product-tracing investigations as an epidemiological tool

The STEC O104:H4 outbreak exemplified the value of product tracing investigations to identify suspected foods in outbreaks (e.g. by linking tracing information to disease clusters). Although successfully applied previously, the methodology is used infrequently thus far. Furthermore, it should be applied as an epidemiological tool for incriminating (or exonerating) suspected food items in outbreaks. It should be further developed and standardised as a tool in analytical epidemiological investigations. In this context it is necessary that legislation for public health or food safety allows for efficient usage of the methodology in food-borne outbreaks.

Economic costs of the outbreak / Burden of illness

This outbreak was unprecedented with respect to the number of HUS cases. Patient treatment is expensive (and long-term consequences are apprehended). Moreover, the outbreak led to costly trade-bans. Similarly, the burden of infectious intestinal diseases in general is substantial. Little is known about the overall burden of these infections, the contribution of the different pathogens to this burden, and the pathogen-specific risk factors. Estimating the costs of this outbreak and of infectious intestinal disease in general (e.g. disability adjusted life years, modelling) is pivotal to guide decision makers in rationally allocating financial resources for research and surveillance of infectious diseases. Population-based data on the pathogen-specific gastrointestinal disease burden are needed to improve targeted prevention and control measures.

Clinical epidemiology

The risk factors for the short-term and the long-term outcome in patient cohorts should be studied. Another research topic is the frequency and determinants of secondary transmission (households etc.) of STEC O104:H4 and other serotypes.

Microbiology and pathogenesis

This outbreak vividly demonstrated the need to rapidly detect and molecularly characterise emerging STEC pathogens. Pathogenesis and evolution of STEC-mediated HUS are incompletely understood.

Microbiological diagnosis

Rapid and innovative methods facilitating early diagnosis of STEC (including emerging strains) should be established. Such approaches should combine molecular detection, culture and isolation of the pathogen and improve both outbreak detection and control and patient management. Methods to type and characterise such pathogens need to be improved. This requires the most advanced sequencing and data management capacities. It is a priority to implement molecular surveillance of STEC, including a central global database, analogous to PulseNet [8], containing typing and virulence data from human STEC isolates linked to STEC databases for animals, food and environment. Methods to detect viable but non-culturable STEC should be improved. It is important to optimise patient management via symptom-based approaches in order to accelerate diagnosis, implement best clinical and infection control practices, and to detect outbreaks as early as possible. Determining the duration of pathogen shedding by patients and quantifying the number of asymptomatic shedders is critical for epidemiological purposes. This should also include enteroaggregative *E. coli* (EAEC) and other diarrhoeagenic *E. coli*.

Pathogenesis and evolution

More evidence is needed on virulence and fitness factors of STEC, and on the mechanisms of their interaction with the host cells and their targets. In particular the roles of Shiga toxins and other toxins, serine proteases, and adhesins, iron uptake systems and growth-promoting factors expressed by these organisms should be delineated. Resistance of STEC to environmental stress, and the environmental factors that promote genetic changes in and spread of STEC virulence factors should be studied (role of phages, plasmids, and other mobile genetic elements). Infection models to study virulence and fitness under different conditions should be refined. Novel host and microbe targets for therapy have to be identified. Another research question relates to the protective host mechanisms including immune response and the role of commensal microbiota. The phylogenetic origin of STEC and their recent evolutionary emergence should be determined. The reservoirs, vectors and transmission

of STEC and other diarrhoeagenic *E. coli* warrant further investigation.

Antimicrobial therapy

A major objective is to determine the effect of antimicrobials on *stx*-phage induction, Shiga toxin production, virulence and resistance gene transfer, and intestinal adherence (in vitro and in animal models). The mechanisms of transfer of antimicrobial resistance, for example extended-spectrum beta-lactamase, via plasmids in response to variable selective pressures should be delineated. The role of biofilm formation in STEC antimicrobial resistance and niche persistence during infection and in the environment should be studied, and the role of antimicrobial therapy in shortening bacterial shedding should be determined.

Food safety, zoonotic and environmental aspects

Food safety

Optimised detection methods are needed for investigations of survival and multiplication of STEC O104:H4 and other STEC in food matrices including fruits and vegetables. Research should be dedicated to the tracing of trading connections. Information systems providing real-time information from different market segments and incorporating the identification of risk factors as well as elements of predictive microbiology should be implemented. Risk profiling and ranking of combinations of foodstuffs and pathogens should be conducted (based on criteria such as microbiology, food processing and consumption, infectious dose, burden of disease). This will allow to identify the optimal sampling and control points along the food chain as well as the most efficient processing steps for decontamination based on hazard analysis and critical control points (HACCP). Decontamination methods for different types of foodstuff should be investigated. These should also take into consideration microbiological characteristics such as biofilm formation.

Zoonotic aspects

Controlled infection experiments with various animal species may provide information about the potential of STEC O104:H4 to cross the species barrier from humans to animals and to establish itself in livestock, wild or pet animals. Studies in animals putatively exposed to STEC O104:H4 during the outbreak or subsequently should elucidate whether the pathogen has already spread to animals and is maintained in animal populations.

Reducing faecal STEC shedding and lowering its prevalence in livestock are promising strategies to protect humans from zoonotic STEC infection. Research is needed to understand host factors that predispose ruminants to STEC infection and shedding such as innate and acquired immunity, intestinal microbiota, genetic background and extrinsic factors such as livestock production systems. Innovative immunisation schemes as well as novel diets and pre- and probiotics against intestinal STEC colonisation in ruminants should be investigated.

Environmental aspects

The risks for products of animal and plant origin becoming contaminated by STEC originating from environmental sources such as soil, water, sewage systems, waste, human and animal shedding should be assessed. Laboratory studies and predictive microbiology may be used for the investigation of survival and multiplication of STEC in different microenvironments, also accounting for possible biofilm formation. Improved sampling and detection methods for environmental samples should be developed. The distribution of environmental STEC should be investigated along the food production chain as well as the horizontal transfer of virulence and antibiotic resistance genes between *E. coli* and other bacteria in these habitats. Research should address the issue of selective pressure by environmental or management factors contributing to the development of niches facilitating the survival or multiplication of STEC and the emergence of new *E. coli* seropathotypes. Decontamination methods for waste water, surface waters and production water such as water used for the irrigation of plants should be assessed.

Conclusions

This massive outbreak had substantial international implications. It exemplified that the landscape of food-borne infections is in flux, that multi-national outbreaks are a reality and that they can occur everywhere, irrespective of food safety standards.

The workshop showed that urgent and challenging research needs exist in the field of STEC and other diarrhoeagenic *E. coli*, and as far as burden of illness is concerned also of other food-borne pathogens. The scientific questions identified need to be further prioritised and strategies to address them should be developed. The workshop delineated interfaces between the working groups. Among those, the most notable are (i) the need to further develop diagnostic methods and to integrate molecular typing into routine surveillance, (ii) to study the pathogenesis, clinical course and new treatment options, (iii) to make use of systematic food tracing data as epidemiological tools, and (iv) to study pathogen evolution among the human host, the environment, and in animal reservoirs.

These research needs must be addressed soon in order to better equip clinicians, microbiologists, public health and food safety authorities for the early detection and efficient control of food-borne outbreaks and to prevent similar events. As it is an obligation of the scientific community to investigate these research questions it is also in the responsibility of national and international funding bodies to fund the respective research programmes.

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Members of the STEC Workshop Reporting Group in alphabetical order:

Corresponding author: K Stark (starkk@rki.de), Robert Koch Institute, Berlin, Germany, R Bauerfeind, Institute for Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Giessen, Germany, H Bernard, Robert Koch Institute, Berlin, Germany, T Eckmanns, Robert Koch Institute, Berlin, Germany, S Ethelberg, Statens Serum Institut, Copenhagen, Denmark, A Flieger, Robert Koch Institute, Berlin, Germany, J Giesecke, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden, M Greiner, Federal Institute of Risk Assessment, Berlin, Germany, H Karch, Institute of Hygiene, University Münster, Münster, Germany, G Krause, Robert Koch Institute, Berlin, Germany, M Mielke, Robert Koch Institute, Berlin, Germany , SJ O'Brien, Institute of Infection and Global Health, National Centre for Zoonosis Research, University of Liverpool, United Kingdom, M Pulz, State Authority for Public Health Lower Saxony, Hannover, Germany, F Scheutz, Statens Serum Institut, Copenhagen, Denmark, A Schielke, Robert Koch Institute, Berlin, Germany, RA Stahl, Department of Medicine, Division of Nephrology, University Hospital Hamburg-Eppendorf, Hamburg, Germany, J Takkinen, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden, PI Tarr, Department of Pediatrics, Division of Gastroenterology and Nutrition, Washington University in St. Louis, United States, RV Tauxe, Centers for Disease Control and Prevention (CDC), Atlanta, United States, D Werber, Robert Koch Institute, Berlin, Germany.

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Letter to the editor: Screening for *Coxiella burnetii* infection during pregnancy: pros and cons according to the Wilson and Jungner criteria

E Leshem (leshem@gmail.com)[1,2]
1. Internal Medicine C and The Center for Geographic Medicine and Tropical Diseases
2. The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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To the editor: I would like to compliment Dr. Munster et al. for their meticulous review of Q fever screening during pregnancy [1]. The recent outbreak of Q fever in the Netherlands highlights the unresolved and poorly understood issues of public health implications, natural history and correct management of Q fever infection during pregnancy.

Q fever infection in pregnant women may pose a considerable risk to medical personnel attending the delivery [2]. Infected animals are known to excrete *Coxiella burnetii* in milk, urine, feces and birth bi-products. Several outbreaks of Q fever were reported in humans exposed to feline parturition [3-5].

In humans, *C. burnetii* infection during pregnancy may result in obstetric complications including spontaneous abortion, intrauterine growth retardation and prematurity, low birth weight and fetal death. Moreover, *C. burnetii* was isolated from the placentas of asymptomatic pregnant women [6]. A classic report described *C. burnetii* infection acquired by an obstetrician following delivery of the fetus and placenta from an infected pregnant woman [2]. This report led to concerns regarding the risk of airborne transmission to medical staff attending pregnant women at delivery who are infected with *C. burnetii*.

The exposure of unprotected medical personnel to high concentrations of a highly infective organism in the placenta probably presents some level of risk (similar to exposure to parturient animals). The high prevalence of asymptomatic Q fever described in pregnant women living in high risk areas suggests considerable exposure of obstetrical staff [7]. This risk should be addressed in guidelines concerning infection control and public health and taken into account when considering screening pregnant women for Q fever in highly endemic areas. Identifying asymptomatic Q fever in pregnant women will allow implementation of infection control measures to prevent infection of obstetric staff during delivery.

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The Communicable Diseases Threat Report now published every week on the ECDC website

T Mollet (Thomas.Mollet@ecdc.europa.eu)[1], E Szegedi[1], C Bogaardt[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

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The Communicable Diseases Threat Report (CDTR) is one of the major outputs of epidemic intelligence at the European Centre for Disease Prevention and Control (ECDC). Prepared by the epidemic intelligence team at ECDC, the public version was formerly only distributed to internal experts, competent bodies of Member States, and international organisations. From now on it will be published on the ECDC website on a weekly basis.

The CDTR is intended for epidemiologists and health professionals in the area of communicable disease prevention and control. It summarises information gathered through epidemic intelligence by ECDC regarding communicable disease threats of concern to the European Union. It also provides updates on the global situation and changes in the epidemiology of communicable diseases with potential to affect Europe, including diseases that are the focus of eradication efforts.

According to the founding regulations of ECDC, the Centre should 'identify, assess and communicate current and emerging threats to human health from communicable diseases' [1]. To fulfil this mandate, the ECDC gathers data from official reports and rumours of suspected outbreaks from a wide range of sources, formal and informal.

Formal reports of suspected outbreaks are received from ministries of health, national institutes of public health, the World Health Organization, academic institutes, including formal notification channels such as the Early Warning Response System [2] and the International Health Regulations [3].

However, in order to ensure a comprehensive picture of health threats to EU security, ECDC gathers epidemic intelligence from informal sources such as media reports, health workers and non-governmental organisations, as well. The objective of epidemic intelligence is to detect, verify, assess and communicate potential or real public health threats as early as possible. Epidemic intelligence enhances the performance of traditional surveillance systems, but also complements them for cross-border alerts and international public health threats.

The ECDC has been implementing epidemic intelligence activities since its creation in 2005 [4]. The early detection at ECDC is in place 24 hours a day including weekends and public holidays.

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