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Rabid dog illegally imported to France from Morocco, August 2011

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In August 2011, a case of canine rabies was notified to the French veterinary services. The dog was a three-month-old puppy illegally imported from Morocco that presented behavioural changes on 1 August and was admitted to a veterinary clinic on 6 August. It died the following day and the body was shortly sent to the national reference centre where rabies was laboratory-confirmed on 11 August. Contact tracing and post-exposure treatment were initiated immediately.

A suspected case of canine rabies was notified to the official veterinary services (Direction Départementale de la Protection des Populations, DDPP) of the district of Vendée, France, on 6 August 2011. The dog was a three-month-old Jack-Russell-type puppy that presented with behavioural changes consisting of progressive sleepiness on 1 August and later, with aggressiveness. The dog was admitted to a veterinary clinic on 6 August and died the following day. Rabies was suspected on admission, and the dog's body was sent shortly after death to the National Reference Centre for Rabies (Institut Pasteur, Paris, France) for biological diagnosis, where rabies was confirmed one day after receipt, on 11 August, by direct immunofluorescence and by virus isolation in tissue culture. The detection of viral RNA by RT-hemi-nested PCR and by real-time PCR was also positive. The viral strain was identified as a lyssavirus belonging to *Rabies virus* species (formerly genotype 1), Africa 1 lineage and genetically closely related to strains isolated in Morocco [1].

Investigation of potential risk to human and animal health

Taking into account the date of onset of signs and the date of death, the maximum viral excretion period, and thus the potential risk of infection transmission, was considered ranging from 18 July to 7 August.

The owners of the dog were a French family who spent holidays in Morocco. They found and adopted the stray dog near Rabat on 11 July 2011. The dog stayed with the family in Morocco until they left on 31 July. They travelled back to France with the dog by car and ferryboat. The dog was not vaccinated against rabies (and was too young to be vaccinated and imported according to European regulations [2]) and was not micro chipped. Conditions required for importation of dogs and entry into the European Union (EU) were not respected [2].

The family drove through Spain during the night without stopping except for fuel and crossed the Spanish-French border on 1 August. In France, the dog did not wander unsupervised but was presented by a child of the family to several persons among her social contacts.

The family members were interviewed by the regional health authorities (ARS) and the DDPP in order to list all known persons and animals that had had direct contact with the dog in France, Spain and Morocco. All persons identified in France were contacted by the physician in charge from the anti-rabies clinic at the university hospital in Nantes to evaluate their individual risk of contamination with regards to the nature and date of contacts, according to World Health Organization (WHO) recommendations [3].

Contact tracing and control measures

In France, three of eight family members who had contact with the dog were considered as category III exposure according to the WHO criteria. All three had been bitten, had transdermal wounds and received post-exposure treatment (PET) consisting of vaccination and rabies immunoglobulin [3].

A total of 19 people among social contacts of the family reported direct contact with the dog. One reported having been bitten and received vaccination and rabies immunoglobulin. A second person had been licked on non-intact skin (skin lesions) and received vaccination

only. Seventeen persons had only category I exposures and did not receive PET [3]. The veterinarian who took care of the dog had previously been vaccinated and therefore only received a booster vaccination. The veterinary nurse who had category I exposures did not receive PET.

No persons had direct contact with the dog in Spain, whereas some relatives and friends in Morocco were identified with at-risk exposures. Health authorities in Morocco were informed and the contact details of the persons affected provided to make sure they would be taken care of. In total, 29 people had direct contacts and six received PET (Table 1).

Another dog, one cat and two kittens had direct contact with the dog at the place of residence of the family in France. The dog was identified by microchip and had previously been vaccinated against rabies. Following the exposure to the rabid puppy, it received a booster vaccination according to French regulations. The cat and both kittens had not been vaccinated nor micro chipped before the contact with the dog and were therefore killed humanely according to the regulations in place.

Table 1

At-risk exposure to the rabid dog and management of patients, France, August 2011

Exposure category[a]	Category 1	Category 2	Category 3	Total
Family members	5	0	3[b]	8
Veterinary staff	1	0	1[c]	2
Social contacts	17	1[c]	1[b]	19
Total	23	1	5	29

[a] According to the World Health Organization criteria [3].

[b] These cases received post-exposure treatment consisting of vaccination and immunoglobulin.

[c] These cases received post-exposure treatment consisting of vaccination.

Discussion and conclusion

No autochthonous human rabies case has occurred in mainland France since 1923. In 2001, France was declared rabies-free for non-flying terrestrial mammals (based on World Organisation for Animal Health (OIE) criteria), following the elimination of fox rabies in 1998. In 2008, the importation of a rabid dog from Morocco resulted in a chain of transmission of canine rabies in France and the loss of the rabies-free status [4]. However, France regained its rabies-free status according to OIE criteria in February 2010 [5].

Since 2001, nine rabid dogs have been illegally introduced in France (Table 2). All but one had been imported from Morocco through Spain. Before the episode described in this report, the most recent case was an adult dog imported from the Gambia to Belgium, and later to France from Belgium. The number of people who received PET after exposure to one of these nine dogs ranged from two to 187 per episode.

Importations in 2004 and 2008 resulted in a high number of post-exposure treatments administered. In 2004, a rabid dog was brought to music and theatre festivals in south western France where 65,000 festival participants were present .A total of 187 people subsequently received PET through a number of anti-rabies clinics in France [6,7].

In 2008, following the secondary transmission of rabies from an imported infected dog, 152 children had to be vaccinated after a rabid dog contaminated in France wandered into a school yard [6].

More recently in 2008, a puppy was found and adopted in Spain by French tourists who had not travelled outside the EU. The rabies strain later isolated from the dog was genetically closely-related to isolates collected

Table 2

Rabid animals illegally imported to France, 2001–2011

Year	French district	Rabid animal	Country of origin	Number of PET
2001	Gironde	Puppy	Morocco	2
2002	Seine Saint Denis	Puppy	Morocco	7
2004	Finistère	Adult dog	Morocco	24
2004	Gironde	Puppy	Morocco	11
2004	Gironde	Adult dog	Morocco	187 [6]
2008	Seine-et-Marne	Adult dog	Morocco	152[a] [4]
2008	Var	Adult dog	Gambia	8[b]
2008	Isère	Puppy[c]	Spain	25 [8]
2011	Vendée	Puppy	Morocco	8

PET: Post-exposure treatment.

[a] Two generations of canine transmission occurred after the importation of the rabid dog. The third case was responsible for most of the human exposures.

[b] Some people exposed in Belgium were taken care of in Brussels.

[c] This dog was found on the highway in southern Spain. The virus was related to Moroccan strains.

in the north of Morocco and in Melilla, an autonomous city of Spain located on the African Mediterranean coast, bordering with Morocco. However, it was not possible to determine whether the dog was directly imported into Spain from these regions during its incubation period or if it was secondarily infected in Spain by another infected animal imported from Morocco or Mellila [8].

These reports illustrate how illegally imported dogs are a health risk in otherwise rabies-free regions and pose an unnecessary strain on the health system due to the required contact tracing. Furthermore, they emphasise the risk of re-introduction and diffusion of the disease in rabies-free countries. Travellers should be informed of animal importation regulations and of the risk of being infected through bites from animals who may present with normal behaviour in the early stages of infection.

The suspicion and identification of rabies in the case described in the current report was carried out early thanks to the awareness of the veterinarian faced with a puppy displaying abnormal behaviour and a history of recent importation from an enzootic country.

Despite the rabies-free status, the maintenance of epidemiological surveillance and medical knowledge both on the human and veterinarian aspects remains critical to insure early recognition of the disease, identification of human and animal at-risk exposure, avoid uncontrolled secondary transmission and implement PET in at-risk people.

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Missed diagnosis of influenza B virus due to nucleoprotein sequence mutations, Singapore, April 2011

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A new influenza B variant was discovered in Singapore in April 2011 during diagnostic testing of a 3-year-old boy with respiratory symptoms. Influenza B virus was isolated from culture and confirmed by standard immunofluorescence testing, but was not detected by the routine, in-house influenza screening reverse-transcription polymerase chain reaction assay that targets the nucleoprotein (NP) gene. Subsequent sequencing investigations demonstrated that several other published assays targeting NP could also fail to detect this novel variant.

Influenza A and B viruses are detectable all year round in tropical Singapore, with overlapping peak incidences of these viruses occurring at approximately the same time, i.e. during calendar weeks 12–30 in the years where such peaks are discernible [1]. Surveillance testing for influenza viruses of samples from patients with influenza-like illness (ILI) obtained from both community clinics and those admitted to hospital has been ongoing in our routine diagnostic laboratory (Molecular Diagnosis Centre, National University Hospital, Singapore) since the emergence of the influenza A(H1N1)2009 pandemic.

On 12 April 2011 (week 18), influenza B was detected by direct immunofluorescence staining (Light Diagnostic Respiratory Viral Screen and Identification DFA Kit, Chemicon International, Millipore, Temecula, CA, USA) and virus culture of a nasopharyngeal swab collected from a 3-year-old boy, who was admitted to our hospital with ILI. The specimen tested negative on our diagnostic influenza A/B duplex real-time, reverse-transcription polymerase chain reaction (rRT-PCR) assay.

This in-house assay was designed to detect all human influenza A and B subtypes and targets the nucleoprotein (NP) gene of influenza B viruses. To investigate the cause of this discrepancy, the NP gene of this influenza B virus isolate was sequenced directly from the clinical sample and compared with that of a patient infected with the routinely detectable, circulating seasonal influenza B virus (B/Singapore/5/2011, GenBank accession number CY093581) isolated during April 2011. Three missense mutations (G158A, G697A, G1465A) and 16 synonymous single nucleotide substitutions, were noted in this putative Singaporean influenza B variant (B/Singapore/1/2011, GenBank accession number CY093580). Further sequencing of the haemagglutinin

Table 1

Primer and probe sequences of influenza B assays presenting mismatches to the influenza B/Singapore/1/2011 virus sequence

Primer/probe name	Sequence (5' to 3')	Nucleotide position (5' to 3')[a]	Sequence orientation	Reference
rRT-PCR				
FluBNA916F20	CC**A**GGGATTGCAGACATTGA	916–935	Forward	This study
FluBNA968U24	TTGTTAG**G**CC**C**TCTGTGGCRAGCA	968–991	Forward	This study
NPB5'705	**A**ACTGGTGTTGCGATC**A**AAGGAGGTGG	717–743	Forward	[8,9]
RT-PCR (including nested)				
FluABC2	A**T**KGC**GC**WYRA**Y**AMWCTYARRTCTTCAWAIGC	1208–1177	Reverse	[11]
FluAB3 (Nested)	GATCAAGTGAKMGRRAGYMGRAAYCC**A**GG	892–920	Forward	[11]
FluB4 (Nested)	CTTAATATGGA**A**ACAGGTGTTGCCATATT	1118–1090	Reverse	[11]

rRT-PCR: real-time, reverse-transcription PCR.
Nucleotides of the primer or probe mismatching the sequence of the possible new variant influenza B/Singapore/1/2011 are in bold.
[a] Based on influenza B virus (B/Singapore/1/2011) nucleoprotein gene, GenBank accession: CY093580.

(HA) and neuraminidase (NA) genes of this virus is planned, to determine if this virus is a possible new variant or whether these changes in its NP gene simply represent the minor antigenic drift phenomenon typical of all influenza viruses.

This NP variation may affect the ability of other PCR-based in-house assays to detect this virus in clinical samples as two of the single nucleotide substitutions (G975A and C978T) were present within the probe-binding region of our in-house assay (Table 1). Therefore we reviewed previously published influenza B assays that target the NP gene. Of the ten influenza B assays identified [2-11], three [8,9,11] had primer and/or probe sequences presenting mismatches that may reduce assay sensitivity. The affected primer/probe sequences are summarised in Table 1.

The probe-mismatch in our in-house assay for this new B/Singapore/1/2011 virus most probably explains the discrepancy with the results from virus isolation and immunofluorescent staining for the case presented, and is a good example of the importance of maintaining virus culture for clinical testing (at least in reference laboratories), despite rapid developments in molecular diagnostic techniques.

To be able to reliably detect the possible new influenza B variant, an additional probe was designed, whilst retaining the previous probe to allow the continued detection of the influenza B/Singapore/5/2011virus that may still be circulating in the Singaporean population. As part of the validation assay for this modified assay, we tested an additional 200 clinical specimens that had been reported as negative for influenza A/B viruses during April 2011, using the previous version of this in-house rRT-PCR screening assay.

Three additional influenza B positive cases were detected that had identical NP sequences to the virus variant isolated from the 3-year-old boy. These three cases included a 75-year-old patient with metabolic syndrome presenting with community-acquired pneumonia and heart failure, a 60-year-old patient who presented with non-neutropaenic febrile illness, and a routine community surveillance specimen (no clinical history provided). All three hospitalised patients were discharged shortly after admission with no clinical complications.

With the use of the modified assay, of the five influenza B viruses detected during April 2011, four were of the possible new variant. Pairwise comparisons between more recent and older reference (vaccine-related) influenza B NP sequences (Table 2) show that the NP sequence of the possible new influenza B variant virus (B/Singapore/1/2011) presents highest similarity to the NP sequence of the most recently circulating B/North Carolina/WRAIR1582P/2009 strain (Victoria lineage). Interestingly, however, when compared to the NP sequences of the older B/Yamagata/16/88 (Yamagata lineage) and of the B/Victoria/2/87 reference strain (Victoria lineage), the possible new influenza B variant virus NP sequence presents higher identity to the B/Yamagata/16/88 (Table 2). As the NP gene is relatively well evolutionarily conserved compared to the HA and NA genes, only additional sequencing of the HA and NA genes from the possible new influenza B variant virus detected in this study, and additional Singaporean influenza B viruses, will clarify whether a novel influenza B variant, not previously seen in Singapore, is emerging, and whether it is of either the B/Victoria/2/87 or the B/Yamagata/16/88 lineage.

The World Health Organization (WHO)'s current seasonal influenza vaccine recommendations for influenza B vaccine composition for the southern hemisphere [12] and for the approaching influenza season in the northern hemisphere [13] are the same. Both include the B/Brisbane/60/2008-like virus, which is of the B/Victoria/2/87 lineage. In China, nevertheless, the B/Yamagata/16/88 lineage viruses have been predominating from September 2010 to January 2011 [13]. China is a popular and frequent destination for many Singaporean Chinese, who have family connections there, so it is likely that influenza B viruses from both the B/Victoria/2/87 and B/Yamagata/16/88 lineages are co-circulating in the Singaporean population. Additional sequencing of Singaporean influenza B viruses will allow an assessment of any potential vaccine mismatch between the seasonal influenza vaccine composition and any possible new variants arising in these locally circulating viruses.

Table 2

Degree of similarity between full-length nucleoprotein gene sequences of Singaporean and reference influenza B viruses, based on pairwise comparisons

	B/Yamagata/16/88 L49385[a]	B/Victoria/2/87 AF100359[a]	B/Florida/4/2006 (Yamagata lineage) CY033879[a]	B/North Carolina/WRAIR1582P/2009 (Victoria lineage) CY069566[a]
B/Singapore/1/2011 CY093580[a]	0.9684	0.9449	0.9851	0.9902
B/Singapore/5/2011 CY093581[a]	0.9730	0.9483	0.9879	0.9925

[a] GenBank accession number.

Conclusions

Our preliminary report highlights two important issues. First, at local level, it is important for diagnostic virology laboratories using molecular techniques to be constantly vigilant about the possibility of emerging novel virus variants that may decrease the sensitivity of their frontline screening assays [14]. This requires the availability of parallel testing using viral isolation by culture. In addition, this report highlights the need for diagnostic laboratories to test any in-house assay that is developed, based on a published method, on their own population of circulating viruses and adapt their primers and probes accordingly, so as to minimise the risk of diagnostic misses.

Second, from a public health perspective, it is essential for diagnostic and research laboratories worldwide to continuously update and share gene sequences of (possible novel) local viruses on public databases. This allows laboratories to develop diagnostic assays for new variants and the *in-silico* assessment of their sensitivity and specificity, i.e. multiple-sequence alignment of the available uploaded sequences against primer/probe sequences.

These two issues are not unrelated. While the clinical management of an individual patient may be adversely affected by a diagnostic 'miss' of a novel, emerging virus, at a global population level, due to the limited time window for the inclusion of novel influenza virus antigens into the WHO recommended composition of the seasonal influenza virus vaccines, timely dissemination of data on such novel virus variants is critical to try to avoid vaccine mismatches as much as possible. Vaccine mismatches can have a serious impact on seasonal influenza morbidity and mortality, particularly in vulnerable groups that rely on vaccines for protection against annual seasonal influenza epidemics worldwide.

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Human cases of West Nile Virus Infection in north-eastern Italy, 15 June to 15 November 2010

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In 2010, for the third consecutive year, human cases of West Nile virus (WNV) infection, including three confirmed cases of neuroinvasive disease and three confirmed cases of West Nile fever, were identified in north-eastern Italy. While in 2008 and 2009 all human cases of WNV disease were recorded in the south of the Veneto region, cases of WNV disease in 2010 additionally occurred in two relatively small northern areas of Veneto, located outside those with WNV circulation in the previous years. WNV IgG antibody prevalence in blood donors resident in Veneto was estimated as ranging from 3.2 per 1,000 in areas not affected by cases of WNV disease to 33.3 per 1,000 in a highly affected area of the Rovigo province. No further autochthonous human cases of WNV disease were notified in Italy in 2010. The recurrence of human cases of WNV infection for the third consecutive year strongly suggests WNV has become endemic in north-eastern Italy.

Introduction

In Italy, the first outbreak of West Nile virus (WNV) infection was reported in 1998 and occurred among horses in the Tuscany region [1]. The virus re-emerged in 2008, when equine and human cases of West Nile neuroinvasive disease (WNND) were notified in the Veneto and Emilia Romagna regions in north-eastern Italy [2]. In Veneto, six clinical cases of WNV infection were identified with disease onset from August to September 2008 and all were from the Rovigo province [3,4]; three further human cases of WNND were notified in Emilia Romagna in September and October 2008 [4,5]. Veterinary and entomological surveillance documented that WNV infection was widespread in the same areas in north-eastern Italy, with notification of 794 equine WNV infections in 251 stables and viral isolation in resident bird species and mosquitoes [2]. Compared to 2008, a trend towards an increasing number of human WNV infections and a spread to a wider geographical area was noticed in 2009 [2], when 17 cases of WNND were notified in northern Italy, including six from Veneto, nine from Emilia-Romagna, and two from the Lombardia region [6-8]. In 2009, we isolated the virus from a blood donor and sequenced its whole genome (Ita09, GenBank accession number GU011992) [7]. Phylogenetic analysis classified the Ita09 isolate as Lineage 1, clade 1a, within the Mediterranean subtype [7], which includes the majority of strains responsible for outbreaks in Europe and in the Mediterranean basin. The full length genome of WNV Ita09 was almost identical to that of WNV isolated from two magpies the year before in the same area [7] and to WNV sequences obtained from mosquito pools collected in Emilia-Romagna in 2009 [9], thus suggesting that WNV might have become endemic in some areas in northern Italy. In these areas, the presence of *Culex pipiens* vector at high density and resident bird species susceptible to WNV infection, like magpies, carrion crows, and rock pigeons, could play an important role in WNV persistence and maintenance during epizootic periods [2].

In this context, in 2010, enhanced National and Regional Surveillance Plans for WNV surveillance were implemented in Italy. This study reports further human cases of WNV infection, who were identified in Veneto in 2010, also in areas north of those affected in 2008 and 2009.

Methods

National Surveillance Plan

During spring 2010, the Ministry of Health published a National Plan for WNND Human Surveillance in Italy, which detailed the activities to be carried out between 15 June and 15 November, the annual period, when the risk for WNV infection is high. In 2010, the surveillance area was enlarged to include municipalities where autochthonous human and veterinary cases of WNV infection had been notified in previous years, as well

as surrounding areas within 20 km from the municipalities. Activities included (i) surveillance of human cases of WNND, (ii) active surveillance of WNV disease and serosurveillance of WNV infection in workers employed in farms where equine cases of WNV infection had been identified, (iii) WNV nucleic acid amplification test (NAAT) screening of blood and haematopoietic stem cell donations in areas under surveillance and, of tissue and solid organ donations, on the whole national territory, (iv) measures for mosquito vector control.

Regional Surveillance Plan

In 2010, the Veneto region implemented the activities of the National Surveillance Plan and intensified the surveillance of human cases of WNV infection by activating an enhanced regional surveillance plan for West Nile fever, as well as seroprevalence studies on blood donors resident in at-risk areas of Veneto.

Case definition of West Nile neuroinvasive disease and West Nile fever

According to the Regional Surveillance Plan implemented in 2010, cases of WNND were defined as being older than 15 years, having fever ≥ 38.5 °C, and neurological symptoms such as encephalitis, meningitis, Guillain-Barré syndrome or acute flaccid paralysis. Cases of West Nile fever were defined as being over 15 years-old, having fever ≥ 38.5°C (or history of fever in the last 24 hours) for a period no longer than seven days occurring from 15 July to 15 November, no history of recent travel to tropical countries, and absence of other concomitant diseases which could account for the febrile illness.

Cases of WNND and West Nile fever were further classified as possible, probable or confirmed. Possible cases of WNND or West Nile fever fulfilled the clinical case definition. Probable cases fulfilled the clinical case definition and at least one of the following laboratory criteria: presence of IgM antibodies against WNV by ELISA; seroconversion by ELISA; fourfold increase of IgG antibodies in acute- and convalescent-phase serum samples (preferably with 15 to 20 days between the two samples) by ELISA. Confirmed cases fulfilled the clinical case definition and at least one of the following laboratory criteria: isolation of WNV from blood and/or, for WNND, from cerebrospinal fluid (CSF); presence of IgM antibodies in CSF by ELISA (for WNND); detection of WNV RNA by RT-PCR in blood and/or CSF (for WNND); detection of increasing levels of IgM and IgG antibodies against WNV by ELISA, confirmed by plaque-reduction neutralisation test (PRNT).

Case laboratory investigations

Possible cases of WNND and West Nile fever occurring in Veneto were referred to the Regional Reference Laboratory. WNV RNA in plasma and CSF samples was detected by using two different real-time RT-PCR methods, targeting WNV lineage 1 [10] and both WNV lineage 1 and lineage 2 [11]; detection of IgM and IgG antibodies against WNV in serum and CSF samples was done by ELISA (WNV IgM capture DxSelect ELISA and IgG DxSelect ELISA kits, Focus Diagnostics, Cypress, California). To confirm the specificity of antibody response, ELISA-positive samples were further tested by PRNT90, with cutoff 1:10 for positive results. PRNT was conducted in a biosafety level 3 laboratory, according to the protocol described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2008 of the World Organisation for Animal Health (OIE) [12].

Active surveillance of stable workers and household contacts

Active surveillance of WNV infection was done for workers employed in farms and for subjects aged ≥15 years residing in farms where equine cases of WNV infection had been identified. Members of households with confirmed cases of WNND or West Nile fever and close contacts of identified human or equine cases of WNV disease were also surveyed. Laboratory tests included detection of IgM and IgG antibodies against WNV in serum and CSF samples by ELISA and confirmation by PRNT, as described above.

Screening of blood and organ donations

In 2010, during the period of surveillance according to the National Surveillance Plan, NAAT screening was performed for all blood and haematopoietic stem cell donations from the Rovigo and Venice provinces, where human cases of WNND had been identified in 2009. In September 2010, following the notification of the first case of WNND, NAAT screening was extended to the Vicenza province. Screening of tissue and organ donations was performed on the whole national territory.

WNV NAAT screening of blood donors was performed by using Cobas TaqScreen West Nile Virus test on Cobas S201 system (Roche Molecular Diagnostics) or the PROCLEIX WNV Assay on PROCLEIX TIGRIS System (Novartis Diagnostics). WNV NAAT-positive cases were confirmed by detection of seroconversion or increasing levels of IgM and IgG antibodies against WNV by ELISA, confirmed by PRNT, as described above.

Screening of tissue and organ donations was done by WNV NAAT using Cobas TaqScreen West Nile Virus test on a Cobas S201 system (Roche Molecular Diagnostics) and by IgM and IgG ELISA, as described above. Laboratory results had to be provided within 72 hours from donation.

West Nile virus IgG seroprevalence study

The prevalence of WNV IgG antibodies was investigated in serum samples collected from 4,450 blood donors (about 6% of blood donation collected during the study period), who were referred to four blood donation centres in different areas in Veneto, in the period from 1 August to 1 December, 2010 (Table). Sample size was determined on the basis of an expected prevalence of 6.8 per 1,000, as determined by a WNV seroprevalence study performed in 2009 [13]. The design and

the results of the seroprevalence study performed in 2009 are available in the paper by Pezzotti et al. [13]. In 2010, serum samples from 48 blood donations per day were collected for WNV IgG antibody testing. In particular, each day, five donations were sampled from Rovigo, centre 3, 12 from Padova, South-East, 16 from Padova, North, and 15 from Verona, choosing serum samples from the first consecutive daily donors who gave their consent for the study. The number of serum samples collected at each centre was proportional to the volume of donations performed in the year 2009. All samples were handled anonymously by technicians and researchers involved in this study.

WNV IgG testing was done and any IgG-positive sample was further evaluated by PRNT for confirmation, as described above. In addition, in IgG-positive samples, the presence of WNV IgM antibodies was also determined as described above.

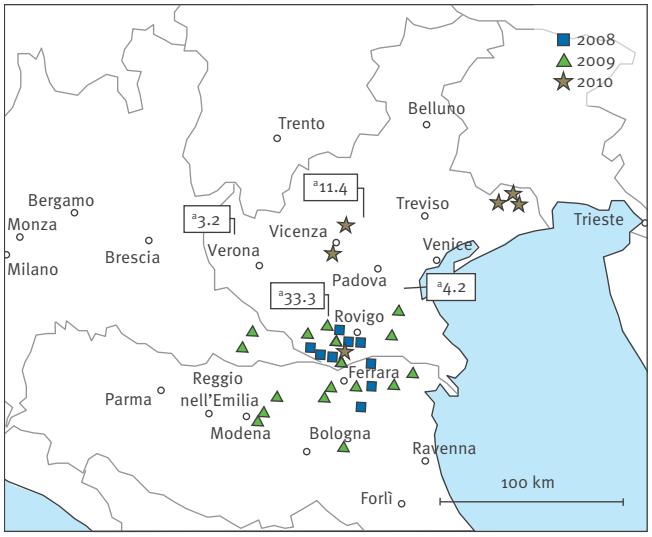
Results

Human cases of West Nile neuroinvasive disease

During the surveillance period in 2010, three males, aged 41–68 years, of 57 possible cases of WNND were confirmed by laboratory tests (all WNND cases were IgM and IgG-positive, confirmed by PRNT, while WNV RNA was undetectable in serum and CSF). Disease onset was at the end of August (two cases from the Venice and Vicenza provinces) and in the middle of October (one case from the Venice province) (Figure). Symptoms included fever, vomiting, headache, altered mental status, and, in one patient, urinary retention. All patients fully recovered.

Figure

Map of north-eastern Italy representing autochthonous human cases of West Nile neuroinvasive disease and West Nile fever notified in Italy, 2008–2010 (n=32)



[a] West Nile virus IgG antibody prevalence per 1,000 blood donors, determined in 2010 in four blood donor centres in Veneto.

A further case of WNND was diagnosed in the Rovigo province in the middle of November 2010. The case, aged in its late 40s, had been hospitalised one month before for viral encephalitis in Romania, in the Braila region, close to areas, where several human cases of WNV disease had been reported in 2010 [14]. This case was therefore considered as an imported case. The patient, who suffered from fever, headache, paraplegia, diarrhoea, myalgia, and pyramidal deficits in both limbs, showed progressive neurological improvement. Laboratory investigation confirmed the presence of IgM and IgG antibodies against WNV.

Human cases of West Nile fever

Of 38 possible cases of WNV infection, three (two males and one female, aged 40–67 years) were positive for WNV IgM and IgG and confirmed by PRNT test, but none of them was WNV RNA-positive. Symptoms included fever, arthralgia, and asthenia in two patients, and fever, headache, abdominal pain, vomit, and diarrhoea in one. One of these patients, after two weeks of fever and arthralgia, showed asthenia, disorientation and retarded movements. All patients fully recovered. Of these three patients, one was resident in the Rovigo province and was the first case of WNV infection identified in 2010, with symptom onset at the beginning of July, one was resident in the Venice province, and one in the Vicenza province (Figure). A further patient from Rovigo, with a retrospective diagnosis of fever, arthralgia, rash, asthenia, and PRNT-confirmed WNV IgG, but IgM-negative, was defined as a probable case.

Active surveillance of stable workers and household contacts

Twelve of 23 household contacts with WNV disease patients were investigated and, among them, an asymptomatic subject resident in Vicenza was found to be WNV IgM and IgG-positive. Active surveillance of WNV infection was also done in all seven workers employed in stables in the Venice province, where equine cases of WNND had been identified [15]. All of them were WNV seronegative.

Surveillance of blood and organ donations

Of 46,045 screened blood donations, two were WNV RNA-positive and were collected in the middle of September from asymptomatic subjects, one resident in the Rovigo province and the other in the Venice province, in the same area where symptomatic cases of WNV infection had occurred. WNV infection was confirmed in both subjects by seroconversion and by real-time RT-PCR targeting WNV lineage 1, thus indicating that these were cases of WNV lineage 1 infection. Due to the low viral load, viral genome sequencing was unsuccessful. No cases of WNV infection were identified among tissue and organ donations.

West Nile virus IgG seroprevalence study in blood donors resident in the Veneto region

Compared to 2009, the seroprevalence investigation in 2010 was extended to more provinces than the Rovigo

province, by adding blood donor centres in the Padova and Verona provinces, as detailed in the Table. In 2010, WNV IgG seroprevalence ranged from 3.2 to 33.3 per 1,000 in the different centres. When compared with 2009, a two-fold increase of IgG WNV seroprevalence was observed in the blood donation centre Rovigo, centre 3, which was included in the study both in 2009 and 2010 (Table).

Distribution of cases of West Nile virus disease

All confirmed autochthonous human cases of WNV disease, including WNND and West Nile fever, identified in Italy in the period from 2008 to 2010 are shown on the map in the Figure. Estimated values of WNV seroprevalence in blood donors from the four centres evaluated in 2010 are also indicated on the map (Figure). While in 2008 and 2009 all human cases of WNV disease were identified in the south of Veneto and in neighbouring Emilia Romagna and Lombardia, in 2010, human cases of WNV disease also occurred in two relatively small areas north of the Venice province, as well as in the Vicenza province in Veneto.

Discussion and conclusions

This study reports six cases of symptomatic and three cases of asymptomatic WNV infection, detected in north-eastern Italy in 2010, an area where WNV seroprevalence was estimated to range from 3 to 33 per 1,000 in 2010. To our knowledge, no further autochthonous human cases of WNV disease were notified in Italy in 2010.

2010 is the third consecutive year that human cases of WNV infection are identified in north-eastern Italy. New areas in the Vicenza and Venice provinces of Veneto, located outside those with WNV circulation in the previous years, have been affected in 2010. A new geographic pattern of WNV spread has also been documented by equine, avian, and entomologic surveillance performed by the Regional and National Reference Centre for Exotic Diseases, which identified cases of WNV infection in horses, resident birds, and mosquitoes in several areas in the north-east (Modena, Treviso, Venice, Verona, Rovigo, and Bologna provinces), the centre (Campobasso province), and the south (Foggia and Trapani provinces) of Italy, some of which have not been previously affected by WNV [15]. Since WNV was circulating in animals in several areas of Italy in 2010, the identification of human cases of WNV disease only in Veneto could be related to the enhanced regional surveillance programme in this region, which was activated in 2010.

Besides autochthonous human cases of WNV infection, we diagnosed a case of WNND imported from Romania, where an epidemic outbreak was ongoing with 57 human cases notified in 2010 [14]. This case report, like the recently described cases of West Nile fever imported from Israel to the Netherlands [16], emphasise the importance of surveillance also for potential imported cases of WNV infection. These imported cases serve as sentinels of the increase in the incidence of WNV disease occurring in 2010 in European and Mediterranean countries, where cases of WNV infection are notified every year, such as Romania (57 human cases in 2010), Hungary (three cases in 2010), Israel (24 cases in 2010), and Russia (480 cases in 2010) [14,16-19]. Moreover, a large human epidemic outbreak with 261 confirmed cases of WNV disease occurred in Greece in 2010, where WNV infection had not been documented in humans before [20]; seven human cases of WNV infection were also confirmed in Turkey [21]. In addition, in 2010, equine outbreaks were reported in Morocco, Portugal, Spain, and Bulgaria [22,23].

In this regard, a recent study on the presence of neutralising antibodies against WNV, detected by neutralisation assay, in intravenous immunoglobulin preparations produced from human plasma samples

Table

Prevalence of serum West Nile virus IgG antibodies in blood donors from the Veneto region, Italy, 2009–2010 (n=6,957)

Province, blood donor centre	Number of samples		Number (%) of WNV IgG antibody-positive samples		Estimated WNV IgG prevalence per 1,000 blood donors [95% CI]	
	2010	2009	2010	2009	2010	2009
Rovigo, centre 1	NR	494	NR	2 (0.40%)	NR	4.0 [0–9.6]
Rovigo, centre 2	NR	1,509	NR	6 (0.40%)	NR	4.0 [0.8–7.2]
Rovigo, centre 3	511	504	17 (3.33%)	9 (1.79%)	33.3 [17.7–48.8]	17.9 [6.3–29.4]
Padova, South-East	719	NR	3 (0.42%)	NR	4.2 [0–8.9]	NR
Padova, North	1,662	NR	19 (1.14%)	NR	11.4 [6.3–16.5]	NR
Verona	1,558	NR	5 (0.32%)	NR	3.2 [0.4–6.0]	NR

NR: not recorded.
WNV: West Nile virus.

collected in Austria, Germany and the Czech Republic demonstrated increasing titres of neutralising antibodies from 2006 to 2010 [24]. Our study also could suggest an increase of WNV IgG seroprevalence in blood donors resident in areas of WNV circulation from 2008 to 2010. The prevalence of WNV IgG antibodies in Veneto ranged from 0.3% in areas not affected by WNV circulation to 3% in affected areas, in line with a previous study performed on solid organ donors in Italy in 2009 [25] and with recent WNV seroprevalence data from Greece [26].

In conclusion, for the third consecutive year, human cases of WNV infection have been identified in north-eastern Italy, suggesting WNV has become endemic in this area. In addition, in 2010, veterinary and entomologic surveillance identified WNV circulation in Italian areas that have not been previously affected by WNV infection. An increased incidence of WNV infection in humans and horses has been also reported in other European and Mediterranean countries. This epidemiological situation urges European countries to enhance surveillance of WNV disease, mosquito control activities, and implementation of measures to prevent transmission to humans through blood transfusion and organ donation.

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Outbreak due to a *Klebsiella pneumoniae* strain harbouring KPC-2 and VIM-1 in a German university hospital, July 2010 to January 2011

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We describe the epidemiology and characteristics of the pathogen and patients (n=7) associated with an outbreak of a carbapenem-resistant *Klebsiella pneumoniae* (CRKP) strain in a German university hospital from July 2010 to January 2011. Species identification and detection of carbapenem resistance were carried out using standard microbiological procedures. Carbapenemases were detected by phenotypic methods and specific polymerase chain reactions (PCRs). DNA fingerprinting profiles were performed with repetitive sequence-based PCR. Medical records of colonised or infected patients were retrospectively reviewed. Antibiotic resistance profiles, PCR-specific amplification products and genotyping demonstrated that the outbreak occurred because of the spread of a single CRKP clone harbouring both KPC-2 and VIM-1. Five of the seven patients had invasive infections with the CRKP strain; the deaths of four of them were directly related to the infection. Early implementation of infection control interventions brought about efficient containment of further cross-transmission. Rapid dissemination of carbapenemase-producing *Enterobacteriaceae* is a serious concern in patient care and is a problem that has emerged in western Europe.

Introduction

Enterobacteriaceae are widespread worldwide and frequently exhibit resistance to extended-spectrum beta-lactamases (ESBLs) [1]. The first-line treatments for infections caused by ESBL-producing strains are carbapenems. The frequent use of carbapenems, however, combined with the transmissibility of resistance determinants mediated by plasmids, transposons and gene cassettes, has contributed to the increase of carbapenem resistance by *Enterobacteriaceae* [2,3]. In countries such as the United States, Israel and Greece, carbapenemase-producing *Enterobacteriaceae* have emerged and the dissemination of these multidrug-resistant pathogens has become a problem in the clinical care of patients and in public health [4-6].

Invasive infections with these organisms have been associated with high rates of morbidity and mortality, due to their resistance to most available antimicrobial agents [7-9]. Patients who are hospitalised for prolonged periods and those with severe underlying disease are at high risk of acquiring these types of pathogens [10].

The Ambler classification describes various classes of carbapenemases [11]. *Klebsiella pneumoniae* carbapenemases (KPC) belong to class A; they are increasingly prevalent in parts of the United States, Israel and Greece and are also an emerging concern in western Europe [12]. Class B metallo-beta-lactamases of the types IMP (imipenemase) and VIM (Verona integron-encoded metallo-beta-lactamase) are common in non-fermentative bacteria and have been recently recognised in *Enterobacteriaceae* worldwide [12]. Since 2003, *Enterobacteriaceae*-producing metallo-beta-lactamases of the VIM type have begun to spread in Greek hospitals [4]. During the first few months of 2010, *K. pneumoniae* strains carrying both VIM and KPC were identified in Greek hospitals [12]. Strains carrying the recently reported New Delhi metallo-beta-lactamase 1 (NDM-1), found throughout India, Pakistan and Bangladesh, have also been rapidly disseminated to Europe and across the globe [13]. OXA-type (oxacillin-hydrolysing) carbapenemases (Class D) are widespread and have been detected primarily in *Enterobacteriaceae* and in *Pseudomonas aeruginosa* [14]. The Ambler classification also describes Class C (AmpC-type beta-lactamases), but these beta-lactamases can only hydrolyse broad- and extended-spectrum cephalosporins, not carbapenems.

Carbapenem-non-susceptible *Enterobacteriaceae* have been sporadically reported in Germany in recent years. In 2008, an outbreak with nine patients affected by a *K. pneumoniae* strain producing a KPC-2 occurred in the southern part of Germany [15]. In 2009 and 2010, two outbreaks in eastern Germany with a *K. pneumoniae* producing KPC-3 were reported, where in 2009 more than 10 patients were affected and in 2010 less than 10 patients (personal communication, Y. Pfeifer, 18 July 2011).

Here we report on an outbreak of a multidrug-resistant *K. pneumoniae* strain harbouring KPC-2 and VIM-1, which affected nine patients in the intensive care unit (ICU) of the Department of General, Visceral and Transplant Surgery in a university hospital in western Germany from July 2010 to January 2011. To the best of our knowledge, this is the first report of the detection of a carbapenem-resistant *Enterobacteriaceae* producing both KPC-2 and VIM-1 outside of Greece.

Methods

Setting

The University Hospital Essen is a German tertiary-care teaching hospital with 1,300 beds; the hospital treats approximately 50,000 inpatients per year. The Department of General, Visceral and Transplant Surgery in the University Hospital Essen is one of the leading liver transplant centres in Europe (158 liver transplants were performed in 2010) and it has a 10-bed ICU. Since January 2000, cultures of nasopharyngeal swabs from all patients on admission to the ICU have been screened once for meticillin-resistant *Staphylococcus aureus*. The natural habitat of *Enterobacteriaceae* is the human gastrointestinal tract and the most practical way to detect carbapenem-resistant Enterobactericeae is to analyse perianal or rectal swabs. Before the outbreak described in this report, no screening for surveillance of carbapenem-resistant *Enterobacteriaceae* was performed in the ICU.

Bacterial strain identification and susceptibility testing

All detected isolates were identified using standard conventional microbial methods (e.g. colony morphologies, pigment production, etc.) [16]. Identity confirmation and susceptibility testing were performed using the semi-automated systems VITEK 2 (bioMérieux, Germany) and MicroScan WalkAway (Siemens, Germany). Minimum inhibitory concentrations (MICs) of meropenem and imipenem were also determined using Etest (AB Biodisk, Sweden) on Mueller-Hinton agar.

Susceptibility results were interpreted using following clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST): ≤2 mg/L for meropenem-susceptibility, ≥8 mg/L for meropenem-resistance, ≤2 mg/L for imipenem-susceptibilty and ≥8 mg/L for imipenem-resistance [17].

Detection of carbapenemases and metallo-beta-lactamases

The modified Hodge test was performed as previously described [18]. Additionally, a combined disk test for imipenem and meropenem was performed with 930 µg ethylene diamine tetraacetic acid (EDTA) and 300 µg 3-aminophenylboronic acid [19,20]. An increase of the zone inhibition diameter ≥7 mm for EDTA and ≥4 mm for 3-aminophenylboronic acid was considered positive.

PCRs were performed for bla_{KPC}, bla_{VIM}, bla_{IMP} and bla_{OXA}-48 genes and amplicons were sequenced as previously described [19,21-23].

Genotyping

Genotyping of all detected isolates was performed by repetitive sequence-based PCR using the semi-automated system DiversiLab (bioMérieux) and the bacterial DNA was amplified with the DiversiLab *Klebsiella* kit. The amplified fragments were separated and fluorescence intensities were detected with a microfluidic chip and a B 2100 bioanalyzer (Agilent Technologies,

Table

Characteristics of patients with carbapenem-resistant *Klebsiella pneumoniae*, university hospital, Germany, July 2010–January 2011 (n=7)

Patient number	Underlying condition	Initial specimen	Infection/colonisation	Antibiotic therapy	Status on hospital discharge
1	Solid organ transplantation	Blood	Bloodstream infection	Tigecycline,colistin	Dead – death unrelated to CRKP
2	Cancer	Pleural fluid	Pleural infection	Fosfomycin	Dead – death related to CRKP
3	Solid organ transplantation	Blood	Bloodstream infection	Tigecycline,colistin	Dead – death related to CRKP
4	Solid organ transplantation	Blood	Bloodstream infection	Colistin,amikacin	Dead – death related to CRKP
5	Solid organ transplantation	Rectal	Colonisation	None	Alive
6	Cancer	Rectal	Colonisation	None	Alive
7	Solid organ transplantation	Tracheal secretion	Bloodstream infection	Tigecycline,colistin, amikacin	Dead – death related to CRKP

United States). The results were illustrated with the web-based DiversiLab software version v.r.3.3.40.

The Pearson correlation coefficient and the unweighted-pair group method with arithmetic mean were used to determine the genetic similarity of the tested samples. A clinical carbapenem-susceptible *K. pneumoniae* strain served as a control, to underpin the discriminatory power of the method. A similarity cut-off of 95% was considered indicative of clonal relatedness, as described previously [24,25].

Results

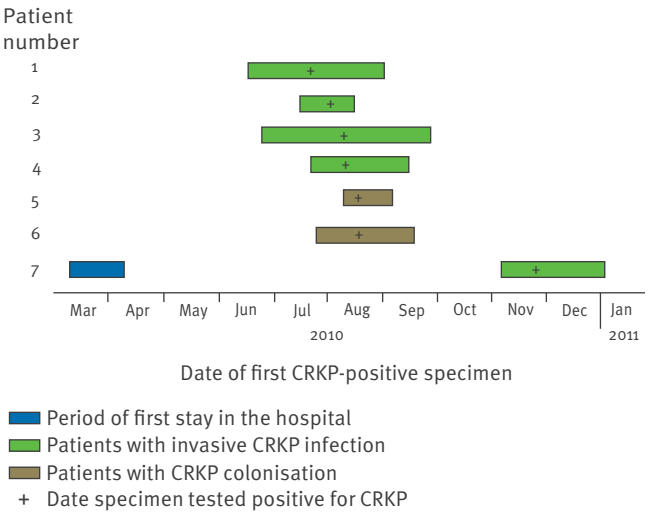
Outbreak description and epidemiological investigations

Carbapenem-resistant *K. pneumoniae* (CRKP) strains were isolated from seven patients hospitalised in the ICU of the Department of General, Visceral and Transplant Surgery of the University Hospital Essen. The age of these patients (five men and two women) ranged from 22 to 72 years. The patient's characteristics are shown in the Table. Bloodstream infections with the CRKP strain occurred in two kidney transplant recipients and two liver transplant recipients. The deaths of three of the four patients were directly related to their CRKP infections.

The first patient (Patient 1) from whom the CRKP strain was isolated (from blood) had received a solid organ transplant in June 2010 and experienced fever and lung infiltrates during the postoperative course. This patient was originally from Germany and there was no evidence that they had travelled to southern Europe, the United States or east Asia in recent years. Nearly two weeks after this first isolation of the CRKP strain was identified, two patients in the ICU (Patients 2 and 3) were found to have invasive infections with this strain, although they were not in the same ICU room as the first affected patient. During the subsequent 12 days, three additional patients (Patients 4–7) tested positive for the CRKP strain, two of whom were colonised only rectally. The synoptic curve of all seven patients is shown in Figure 1.

Figure 1

Synoptic curve of patients with carbapenem-resistant *Klebsiella pneumoniae*, university hospital, Germany, July 2010–January 2011 (n=7)



CRKP: carbapenem-resistant *Klebsiella pneumoniae*.

A patient from Greece, who had been previously hospitalised in Greece (Patient 7), received a solid organ transplant in March 2010, three months before the *Klebsiella* outbreak occurred. At that time, this patient had *Enterobacter cloacae* bacteraemia, but during this period, no CRKP strain was isolated from this patient or from other ICU inpatients. At the end of March, the patient was discharged, in a good medical condition. In November 2010, this person was again hospitalised at the University Hospital Essen. The patient had travelled to Greece between the two hospitalisations in Germany and had had contact with the healthcare system in Greece during the interval between hospitalisation. The patient also developed bacteraemia with the CRKP strain and died at the beginning of January 2011.

Antimicrobial identification and susceptibility

All studied isolates were identified as *K. pneumoniae*. They exhibited the same multidrug-resistant profile, which showed resistance to all beta-lactams, including imipenem and meropenem (MIC ≥8 mg/L), in the semi-automated systems. The Etest yielded MIC values of ≥32 mg/L for imipenem and meropenem. All CRKP isolates, with the exception of one isolate from Patient 7, were found to be susceptible to colistin, tigecycline and fosfomycin according to the EUCAST breakpoints. Susceptibility testing of this isolate from this patient demonstrated resistance to colistin (MIC >2 mg/L) and fosfomycin (MIC >32 mg/L).

Carbapenemase characterisation

All isolates were screened for carbapenemase production using phenotypic and molecular methods. The modified Hodge test yielded positive results for all isolates, indicating the presence of a carbapenemase. However, the combined disk test with EDTA for the detection of metallo-beta-lactamases was positive only in one isolate and only for meropenem. The combined disk test with 3-aminophenylboronic acid for the detection of KPC enzymes was negative for all isolates. By PCR and subsequent sequencing, bla_{KPC}-2 and bla_{VIM}-1 genes were found in all isolates.

DNA fingerprinting

As depicted in Figure 2, semi-automated repetitive sequence-based PCR demonstrated that all VIM-1-producing and KPC-2-producing *K. pneumoniae* isolates had indistinguishable band patterns, with a percentage similarity higher than 95%, which indicates clonal relatedness. The CRKP strain from Patient 7, which was the last isolate detected during the outbreak, was also clonally related to the other isolates.

Infection control measures and interventions

After the second and third CRKP isolates were detected in routinely collected samples, an outbreak control team was organised and infection control strategies were established and implemented (Box).

All CRKP-positive patients were isolated in single-bed rooms, where strict contact precautions were maintained. Barrier nursing (i.e. use of gowns, masks and gloves) was used and contact precautions were also implemented for patients who had shared a room with a CRKP-positive patient. The transfer of CRKP-positive patients to other units or hospitals was discontinued.

To identify the prevalence of CRKP colonisation/infection, samples for cultures from various body sites (nasopharynx, urinary tract and rectum) were collected once a week from all ICU inpatients and all patients who had been moved to a different ward in the hospital. A patient was considered to be CRKP negative if all cultures of samples collected from the three body sites were found to be negative on three different days with a two-day interval between sampling. All surveillance samples were cultured on an internally validated selective medium containing imipenem, vancomycin and amphotericin B for specific detection of carbapenem-resistant Gram-negative bacterial rods. Furthermore, environmental sites were investigated for CRKP contamination. In total, 32 patients and 68 environmental sites were screened during the outbreak period. The environmental samples were taken from medical devices (e.g. X-ray apparatus and syringe pumps), personal computers, telephones, door handles, floors and various other surfaces in the bathrooms or offices in the ICU. Screening of cultures from samples collected from all inpatients in the ICU detected two additional colonised patients (Patients 5 and 6) who had not previously been found to harbour CRKP and had not initially been treated with contact precautions. Environmental sampling demonstrated the presence of *Acinetobacter baumannii* and *Staphylococcus aureus* on medical equipment, but no carbapenem-resistant *Enterobacteriaceae* were detected.

All ICU personnel, including cleaning staff and physical therapists, were instructed about standard hygiene procedures such as appropriate hand hygiene. In addition, the number of nursing staff in the ICU was increased during the outbreak period so that the increased nursing effort for isolated patients could be managed.

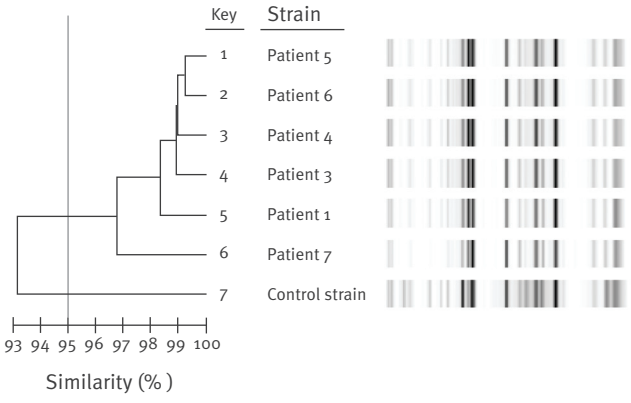
Discussion

The epidemiological and molecular investigations showed that a single *K. pneumoniae* strain that produced both VIM-1 and KPC-2 was responsible for the outbreak. To the best of our knowledge, the occurrence of VIM-1-positive and KPC-2-positive *K. pneumoniae* isolates has previously been reported only in Greece [26,27].

In July 2010, the first patient developed an infection with the VIM-1- and KPC-2-positive CRKP outbreak strain and cross-transmission occurred thereafter. In November 2010, Patient 7, who had been an inpatient in March 2010, was again hospitalised in the CRKP-affected ICU after being discharged from a Greek hospital. The patient also had bacteraemia caused by the CRKP outbreak strain. Our investigations yielded no proof that the CRKP strain originally came from another country; in our opinion, it might be possible that the strain could had been transferred to the German hospital in March 2010 during the first hospital stay of the patient and had remained undetected until July. As described in several previous reports, importation of carbapenem-resistant strains into other European

Figure 2

DNA fingerprinting of carbapenem-resistant *Klebsiella pneumoniae* isolates by repetitive sequence-based PCR, university hospital, Germany, July 2010–January 2011 (n=6)[a]



The dendrogram and gel image demonstrate strain clustering. The horizontal bar on the bottom left indicates the percentage similarity within the strains. A cut-off of 95% similarity (vertical line) was chosen for determination of clonal relatedness. A clinical carbapenem-susceptible *K. pneumoniae* strain served as a control.

[a] One carbapenem-resistant *Klebsiella pneumoniae* isolate (from Patient 2) could not be saved for DNA fingerprinting.

Box

Interventions implemented for control of an outbreak due to carbapenem-resistant *Klebsiella pneumoniae* in an intensive care unit, university hospital, Germany, July 2010–January 2011

- Isolating patients in single rooms or isolating cohorts with the same CRKP species and same resistance profile
- Obtaining targeted surveillance samples from all inpatients and contact patients
- Stopping the transfer of CRKP-positive patients to other units or hospitals
- Increasing the number of nursing staff in the intensive care unit during the outbreak period
- Instructing personnel, including cleaning staff and physical therapists, about standard hygiene procedures (e.g. appropriate hand hygiene)
- Disinfecting rooms with 3% nebulised Pentakalium-bis(peroxymonosulfate)-bis(sulfate) and cleaning rooms with 0.5% Pentakalium-bis(peroxymonosulfate)-bis(sulfate) after discharge of a CRKP-positive patient

CRKP: carbapenem-resistant *Klebsiella pneumoniae*.

countries by patients who had been previously hospitalised in Greece is not uncommon [15,28-32].

Due to the limited number of patients, we did not carry out a case–control study. We did notice, however, that recipients of solid organ transplants were particularly affected by the CRKP strain during the outbreak and developed invasive infections. As has been reported by others, such transplant recipients are frequently affected by carbapenem-resistant *Enterobacteriaceae*. For example, a study from a liver transplant centre in France found that, in 2003 and 2004, eight patients were infected with an imipenem-resistant, VIM-1-producing *K. pneumoniae* isolate; seven of these patients had undergone liver transplantation [32]. The index patient in that outbreak was a liver transplant recipient, who had been transferred from Greece to France. In addition, in the first described German outbreak with a KPC-2-positive *K. pneumoniae*, two of nine affected patients had received solid organ transplants [15].

Recipients of solid organ transplants are routinely and extensively given broad-spectrum anti-infective agents as prophylaxis or targeted therapy during the preoperative, perioperative and postoperative periods. Broad-spectrum antibiotic therapy exerts a selective pressure towards resistant organisms and affects the normal body flora. Furthermore, transplantation, immunosuppressive therapy and intensive care medicine, including use of medical devices, allow pathogens to colonise or infect patients. Therefore, patients who have received solid organ transplants may be at higher risk of acquiring carbapenem-resistant *Enterobacteriaceae* in outbreak situations than are inpatients who have not received such transplants.

Laboratory identification of carbapenemase-producing *Enterobacteriaceae* is challenging. Several bacteria produce carbapenemases and there is a high degree of diversity of these enzymes. As shown in our study and reported by others, the negative results of EDTA or boronic acid-based testing of the CRKP isolates may be attributed to the masking effect of the co-produced carbapenemases [26,27]. These concerns must also be considered when carbapenemase-producing isolates are detected. Microbiology laboratories without the necessary expertise are urged to send potential strains to reference laboratories for appropriate testing. It should be noted that early detection of carbapenem-resistant isolates is of utmost importance in allowing adequate antimicrobial therapy to be initiated and in avoiding cross-transmission.

Containment of the outbreak began after the establishment of an outbreak control team that initiated infection control interventions. It is well known that hospital staff are potential vectors for transmission of resistant organisms, with documented carriage on hands or clothing [33]. However, we decided not to screen hospital staff for hand carriage because no legal guidelines exist in Germany on how to deal with the affected persons. However, additional cross-transmission was then prevented by strict patient isolation, intensified hand disinfection and the routine analysis of targeted surveillance cultures.

This outbreak led us to screen immunosuppressed patients or organ transplant recipients in ICUs for carbapenem-resistant *Enterobacteriaceae* by taking rectal swabs once a week and to monitor the course of colonisation or infection. We strongly recommend the use of perianal surveillance cultures, especially for patients who have been previously hospitalised in countries in which carbapenemase producers are widespread (e.g. Greece, Israel and the United States). Preventive contact precautions should be carried out for such patients until the results of microbiology studies have been obtained. In France, for example, carbapenemase-producing *Enterobacteriaceae* emerged in 2004 and the French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS), recently recommended reinforcement of screening and control measures at national level when cross-border transfer of patients occurs [34].

For the microbiological analysis of targeted surveillance cultures, we suggest using a selective medium containing imipenem, vancomycin and amphotericin B so that the growth of Gram-positive and carbapenem-susceptible Gram-negative bacteria and even fungi in rectal flora can be avoided. Other authors have reported that media containing cefotaxime or ceftazidime are also feasible for the detection of multidrug-resistant *Enterobacteriaceae* [35].

In summary, a VIM-1- and KPC-2-positive *K. pneumoniae* strain was responsible for an outbreak that affected seven patients in one ICU.

Rapid dissemination of carbapenemase-producing *Enterobacteriaceae* is a serious concern in clinical patient care and these pathogens are now also present in western Europe. Therefore, using surveillance cultures and initiating strict hygiene procedures is mandatory for the prevention and early detection of carbapenem-resistant *Enterobacteriaceae* in units where high-risk patients receive care.

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ESCAIDE 2011 - call for late breakers

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The 2011 European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) will take place in Stockholm, Sweden, from 6 to 8 November.

As last year, a so called 'Late Breaker' session will be organised at ESCAIDE. The call to submit abstracts to the 'Late Breaker' session opens on 29 August. Please visit the conference website, www.escaide.eu, to read more about the eligibility criteria for abstract submission. The deadline for submitting abstracts to the 'Late Breaker' session is Friday 23 September.

Programme details and conference registration instructions are available on the ESCAIDE website. As in previous years, it is anticipated that the conference will be accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide CME credits. For further information, contact: escaide.conference@ecdc.europa.eu