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Cases of community-acquired meticillin-resistant *Staphylococcus aureus* in an asylum seekers centre in Germany, November 2010

S Dudareva (DudarevaS@rki.de)[1,2], A Barth[3], K Paeth[3], A Krenz-Weinreich[4], F Layer[5], Y Deleré[6], T Eckmanns[6]

1. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. Postgraduate Training for Applied Epidemiology, Robert Koch Institute, Berlin, Germany
3. Public Health Department, City of Neumünster, Germany
4. Private practice Dres. Krenz-Weinreich and Schulze, Plön, Germany
5. National Reference Centre for Staphylococci, Robert Koch Institute, Wernigerode, Germany
6. Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany

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In an asylum seeker centre in Schleswig-Holstein, a resident was diagnosed with furuncle caused by a Panton-Valentine leukocidine (PVL)-positive community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA). As a result of active case finding, 232 of 427 persons (54% of all residents) were screened for MRSA and two further PVL-positive CA-MRSA cases were identified.

In mid-November 2010, around three weeks after arrival at an asylum seeker centre located in Neumünster, central reception centre of Schleswig-Holstein, Germany, a resident originating from Somalia (case 1) was diagnosed with furuncle caused by a PVL-positive CA-MRSA. On the same day, the resident was moved from a four-bed room to isolation with separate bathroom and toilet.

Following the identification of this case, we decided to perform active case finding and to collect nasal swabs from contacts of case 1 and other residents of the centre. The objectives of the investigation were to identify further cases and to stop possible transmission of PVL-positive CA-MRSA. We were able to reach a high number of residents due to blood collection and a vaccination campaign that were taking place at the same time in the course of a measles outbreak in the centre. In parallel, an intervention plan was developed in order to stop possible transmission. Following the active case finding, four more CA-MRSA cases were identified. Two of these were PVL-positive.

In recent years, emerging community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have become a public health problem, and rational strategies for the control of staphylococcal colonisation and infections in non-hospital settings are required [1]. Compared to the general population, close household contacts of the infected person, prison inmates, military recruits and children attending childcare centres are more frequently colonised with CA-MRSA [2-4]. A Swiss study identified non-Swiss/non European Union citizenship and a history of staying in a collective housing facility as risk factors for CA-MRSA infection [5].

The definition of MRSA as CA-MRSA is primarily based on epidemiological criteria (e.g. acquired outside the hospital setting, lack of risk factors typical for infections with hospital-associated MRSA).Cases of CA-MRSA are frequently positive for the genes encoding the Panton-Valentine leukocidine (PVL), a two-component leukolytic toxin associated with skin and soft-tissue infections, typically furunculosis [1,4]. Although the infection is usually confined to skin and soft tissues, systemic complications, including necrotising pneumonia, have been reported [1]. Furthermore, a number of CA-MRSA represent clonal lineages which are different from those of widely disseminated hospital-associated MRSA.

In the asylum seekers centre described here, residents stay from six weeks up to several months, on average two months, before they are placed in other asylum seekers' centres in Schleswig-Holstein. The center consists of former military barracks and the residents are housed in large shared rooms (four to six people) and share a bathroom and on average a toilet per eight rooms. Upon arrival, each resident receives a hygiene-package, containing towel, soap, body lotion, toothbrush and toothpaste. In the central laundry service, everyone can hand over their clothes and towels. Bed linen is centrally washed at 75°C with disinfectant. Meals are served in a central dining room.

Case finding and microbiological investigation

For our investigation a case was defined as a person residing in the asylum seeker centre during the time of the investigation (between 19 and 22 November 2010) and who was diagnosed with CA-MRSA by laboratory tests. Contacts were defined as persons living in the same bedroom or belonging to the same family and playing together or having close physical contact with the case and residing in the asylum seeker centre during the time of the investigation.

Two days after the initial case was identified, we started convenience sampling and we collected nasal swabs from the contacts of MRSA cases and from the majority of those coming to blood collection and/or vaccination. In addition, for MRSA cases, swabs were taken from the throat, axilla and groin and, where applicable, from wounds. The collected samples were sent for diagnostic tests to a private laboratory in Plön and results were received on a daily basis. Sterile cotton-tipped swabs were used in screening. Plating was on ID-Agar-Plate (BioMérieux, Marcy l'Etoile, France). Suspected colonies were tested by agglutination, thus *S. aureus* was confirmed by Slidex Staph Plus® (BioMérieux, Marcy l'Etoile, France). If the agglutination test was positive, oxacillin resistance was tested by PBP2® (Oxoid, Basingstoke, Hants, United Kingdom). Samples positive

Table 1

Decolonisation procedures for PVL-positive CA-MRSA cases, asylum seekers centre, Schleswig-Holstein, Germany, November 2010

Procedure	Specification
• Washing skin and hair with octenidin-based solution	daily
• Application of mupirocin nasal ointment	three times daily
• Gurgling using 0.1% chlorhexidine solution	three times daily
• Hand disinfection with an alcohol-based antibacterial hand sanitizer	several times daily
• Disinfection of personal items (e.g. comb, shaver, glasses, jewellery) with an alcohol-based antimicrobial cleanser	daily
• Disinfection of bathtub or shower floor and of smooth surfaces with an alcohol-based antimicrobial cleanser	daily
• Changing and washing (at least at 60°C using a laundry detergent) of towels, bed lines, underwear and clothing	daily
• Using new toothbrush	at each tooth brushing
• Using new shoes (with socks)	for the decolonisation period

CA-MRSA: community-acquired meticillin-resistant *Staphylococcus aureus*; PVL: Panton-Valentine leukocidine.

Table 2

Cases of CA-MRSA and PVL-positive CA-MRSA cases among asylum seekers, Schleswig-Holstein, Germany, November 2010

Country of origin	Number of residents	Residents tested		CA-MRSA positive cases	PVL-positive CA-MRSA cases
		Number	(%)	Number	Number
Serbia	74	60	(81)	3	2
The former Yugoslav Republic of Macedonia	44	26	(59)	1	0
Somalia	14	11	(79)	1	1
Other	295	135	(46)	0	0
Total	427	232	(54)	5	3

CA-MRSA: community-acquired meticillin-resistant *Staphylococcus aureus*; PVL: Panton-Valentine leukocidine.

Table 3

Demographical information of the PVL-positive CA-MRSA cases and laboratory results, asylum seekers centre, Schleswig-Holstein, Germany, November 2010

Case	Age (years)	Country of origin	Days spent in the centre[a]	Persons sharing the room	Positive sites	Resistance	Virulence factors	SPA type, clonal complex
Case 1	19	Somalia	23	4	N, T, Ax, W	P, A, AS, Ox, Ce, Te, Co	lukPV+	to21, CC30
Case 2	4	Serbia	41	6	N	P, A, AS, Ox, Ce[b]	lukPV+ etd+	to44, CC80
Case 3	8	Serbia	41	6	N, T	P, A, AS, Ox, Ce, Te, F	lukPV+ etd+	to44, CC80

A: amoxicillin; AS: ampicillin/sulbactam; Ax: axilla; Ce: cefaclor; Ci: ciprofloxacin; Cl: clindamycin; Co: co-trimoxazole; CA-MRSA: community-acquired meticillin-resistant *Staphylococcus aureus*; E: erythromycin; F: fusidic acid; G: gentamicin; L: linezolid; N: nose, Of: ofloxacin; Ox: oxacillin; P: penicillin; PVL: Panton-Valentine leukocidine; R: rifampicin; T: throat; Te: tetracycline; Ti: teicoplanin; V: vancomycin; W: wound.

[a] Days from arrival in the centre until the day of laboratory confirmation of PVL-positive CA-MRSA.

[b] The resistance pattern is incomplete (F, L, V, Ti were not tested).

for MRSA were sent to the German National Reference Laboratory for *Staphylococci* in Wernigerode for further characterisation and typing. A multiplex-PCR for the presence of genes specific for particular CA-MRSA-clones was also performed [6]. Nasal swabs were collected again from the three PVL-positive CA-MRSA cases who had undergone decolonisation procedures and were also sent to the private laboratory.

Information on previous hospitalisations, chronic illnesses and previous use of antibiotics was gathered for all MRSA cases.

Intervention for CA-MRSA cases

In order to control further transmission, persons colonised by PVL-positive CA-MRSA had to follow a stringent decolonisation strategy. The decolonisation protocol used was based on existing national hospital-associated MRSA decolonisation measures [7] and were agreed on in a teleconference with the participation of national MRSA experts and the local health authority. Non-PVL-positive CA-MRSA cases were not entitled for decolonisation at this setting. The measures were implemented upon receipt of the laboratory confirmation for a duration of five days and are summarised in Table 1.

In addition, cases were instructed to enhance hand hygiene and to minimise contact with other residents of the centre during the five days of the decolonisation process. The persons were not allowed to be relocated to other centres prior to successful decolonisation.
To determine the success of decolonisation measures, nasal swabs were taken from these persons three days after the end of the procedure and further swabs shall be applied six days and 12 months after decolonisation. In case of a positive sample three days after the decolonisation process, decolonisation had to be repeated. In case of a positive sample after a second decolonisation process, systemic antibiotics should be applied.

Results

At the time of the investigation, 427 residents from 18 nations were in the centre. The median age was 23 years (mean age 22.7, SD ±14.1 years) and the male to female ratio was 1.5. Samples were collected from 54% of the residents (232/427); five persons were positive for MRSA. Epidemiological criteria and molecular typing results revealed them as CA-MRSA. From those, PVL-positive CA-MRSA were confirmed in three cases. Results on tested persons and laboratory results are presented in Table 2.

PVL-positive CA-MRSA cases

Three PVL-positive CA-MRSA cases were identified. Except case 1, no other case had wounds or abscess on the skin and none reported a contact with case 1. The demographics of the cases and the laboratory results are summarised in Table 3.

A different CA-MRSA strain was identified in each of the three cases. The CA-MRSA strain of case 1 was assigned to CC30, which is prevalent in South East Asia, Oceania, United States of America, United Kingdom, Denmark and the Baltic countries. Sporadic cases are also described in other European countries [8]. Cases 2 and 3 (siblings) belonged to the main European clone ST80, but the resistance pattern differed.

All three cases reported previous hospitalisation in their home countries: case 1 in 2005 for surgery, case 2 in 2009 for conservative treatment, case 3 in 2006 for conservative treatment and in 2008 for surgery. None of the cases reported previous use of antibiotics. Four other family members living in the same room (for cases 2 and 3) and three roommates (for case 1) were swabbed and were negative for MRSA. Eight known play partners for case 2 and 3 were swabbed and were also negative.

Results of the decolonisation

Nasal swabs were taken from the three treated persons three days after decolonisation. For case 3, the swabs taken three and six days after the first decolonisation were negative, whereas cases 1 and 2 had a negative swab three days after repeated decolonisation. For the case 1 and 2 we do not have the information about the results for the swabs collected after six days. The cases were later transferred to other centres and the respective authorities were informed about the further control tests necessary.

Discussion

We conducted the investigation and control of cases of CA-MRSA in an asylum seeker centre in Germany. In our investigation, five cases of CA-MRSA were detected. Of those, three were PVL-positive CA-MRSA, however all three were different in respect of the resistance pattern. Furthermore two isolates were assigned to spa-type t044 (siblings, cases 2 and 3), one isolate presented spa-type t021 (case 1). No transmission in this setting was identified. Regarding the siblings (cases 2 and 3) probably both children were carrying the same CA-MRSA ST80 strain, although the resistance profiles differed in respect of tetracycline and fusidic acid. Genetic studies indicate that the resistance to tetracycline (*tetK*) and fusidic acid (*far1*) is located on a 28 kb plasmid and the loss of this plasmid in case 2 may explain the two different resistance profiles.

Although residents live on average with four more persons per room and an average of 40 persons share the same bathroom and toilet, these factors apparently did not facilitate transmission within the centre. This might be related to the fact that the residents only live in the centre for a relatively short period (on average two months) and that they tend to use personal hygienic items and there are no shared towels in bathrooms and toilets.

Information about the prevalence of CA-MRSA in non-hospital communities is limited. In healthcare associated *Staphylococcus aureus* isolates in Europe, the proportion of MRSA varies from below 1% in Northern Europe to more than 40% in Malta, Estonia and Portugal [9]. In healthcare associated *Staphylococcus aureus* isolates in Serbia, the proportion of MRSA is reported as 64% [10]. No published information about Somalia was found.

Outbreaks of skin infections associated with CA-MRSA within a defined community have been reported previously. Several published outbreaks occurred in children day care centres, sport teams or among military recruits [4]. A furunculous outbreak related to *lukS-lukF* positive *Staphylococcus aureus* in a German village has been successfully terminated with stringent decolonisation procedures [11]. However, re-colonisation has been observed within several weeks after successful decolonisation in up to 22% of cases [12]. Therefore, following up the cases is of great importance.

Although no further transmission was observed, asylum seeker centres are settings where the appearance of PVL-positive CA-MRSA* should be treated with special attention and protocols for decolonisation should be in place. The control strategy described in our report may be helpful for others who have to cope with PVL-positive CA-MRSA* infections in non-hospital settings.

***Authors' correction:**
At the request of the authors, the following change was made on 10 February 2011: 'CA-MRSA' was replaced with 'PVL-positive CA-MRSA' in the last paragraph.

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Usefulness of *porA* sequencing in distinguishing sporadic and linked cases of serogroup B invasive meningococcal disease in Suffolk, United Kingdom, December 2009 to January 2010

A Varadarajan (abrajan@doctors.org.uk)[1], T Sundkvist[1], N Jayatilleke[1], C Williams[1], S J Gray[2], A D Carr[2], E B Kaczmarski[2]
1. Norfolk, Suffolk and Cambridgeshire Health Protection Unit, Health Protection Agency, Ipswich, Suffolk, United Kingdom
2. Meningococcal Reference Unit, Health Protection Agency, Manchester, United Kingdom

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A cluster of three fatal cases of invasive meningococcal disease due to *Neisseria meningitidis* serogroup B in a town in Suffolk, United Kingdom, during December 2009 to January 2010 was reported to the local Health Protection Unit. This paper describes the investigation undertaken to identify any potential epidemiological links among the cases, to determine if this was an outbreak and to consider whether to implement community-wide interventions and control measures. Case epidemiological information in addition to serogroup and genosubtyping (*porA* gene sequencing) data of the infecting organism was gathered on all cases in this reported cluster. Genosubtyping was also retrospectively requested for all serogroup B cases confirmed in Suffolk during 2009. Extensive investigation failed to establish an epidemiological link among the cluster of fatal cases of serogroup B invasive meningococcal disease in Suffolk. By demonstrating a number of distinct strains, the genosubtyping of isolates proved to be useful in the public health management of this incident by serving to exclude a community outbreak and preventing unnecessary mass chemoprophylaxis.

Introduction

Invasive meningococcal disease is caused by *Neisseria meningitidis*, which is a normal inhabitant of the human nasopharynx. Transmission from person to person occurs by contact with droplets or secretions from the upper respiratory tract. Meningococci are classified into serogroups based on capsular polysaccharide antigens: there are at least 13 serogroups, of which serogroups A, B, C, W135 and Y account for the majority of the invasive meningococcal disease worldwide [1]. In the past, serogroups B, C and Y were the most common in the United Kingdom (UK). Following the introduction of the meningococcal serogroup C conjugate vaccine into the UK routine immunisation programme in November 1999, the number of cases of invasive meningococcal disease caused by serogroup C fell by over 90% [2]. Currently, serogroup B strains account for around 90% of laboratory-confirmed cases submitted to the Health Protection Agency.

Invasive meningococcal disease, although relatively rare, is a life-threatening infection with case fatality rates of about 10% [3,4]. The annual rate of invasive disease in the United Kingdom (UK) ranges from two to six per 100,000 population, with a higher incidence in the winter months. The incidence of meningococcal disease is highest in infants under one year of age, followed by children aged one to five years. The next highest incidence is seen in young people aged 15 to 19 years [1,3].

Between 14 December 2009 and 5 January 2010 the local Health Protection Unit in the county of Suffolk, United Kingdom, received reports of four fatal cases of suspected meningococcal disease associated with a Suffolk town. According to mid-2007 estimates, the population of the town was 121,047 and that of Suffolk was 709,409 [5].

All cases were notified within 24 hours of onset of symptoms. The local Health Protection Unit launched an investigation for potential epidemiological links between the cases, to identify if it might be an outbreak and to consider whether to implement interventions and control measures.

The aim of this paper is to demonstrate the usefulness of *porA* sequencing in distinguishing sporadic and linked cases in the investigation of a cluster of fatal cases of serogroup B invasive meningococcal disease.

Methods

We designated this group of cases as a cluster, in line with the United States Centers for Disease Control and Prevention (CDC) definition of a cluster as 'an aggregation of cases of a disease or other health-related conditions which are closely grouped in time and place'

[6]. This definition does not assume that the number of cases is more than expected, nor that expected number is known.

Case definition

Confirmed case

A confirmed case was defined as an individual with a clinical presentation of invasive meningococcal disease, of any age with a place of residence or overnight stay (during the seven-day incubation period) in the local authority area of the town in Suffolk, with onset of illness on or after 1 December 2009 and with a laboratory confirmation of serogroup B.

Probable case

A probable case was defined as an individual with a clinical presentation of invasive meningococcal disease (without laboratory confirmation of serogroup B), of any age, with a place of residence or overnight stay (during the seven-day incubation period) in the local authority area of the town in Suffolk and with onset of illness on or after 1 December 2009.

Clinical presentation

The early symptoms of meningococcal disease may be non-specific and different in infants and older children. Symptoms in infants include fever, floppiness, high-pitched crying and sometimes vomiting. Older children and adults may present with fever, increasing headache, neck stiffness, photophobia, nausea and vomiting. Some may present with a distinctive petechial rash.

Case identification and investigation

The first fatal case was a young child (Case 1) with fulminant septicaemia, who was notified to the local Health Protection Unit on 14 December 2009 as a probable case of invasive meningococcal disease. On 31 December 2009 two further fatal probable cases (Cases 2 and 3, both young children) were notified to the local Health Protection Unit. All three cases were from the same town in Suffolk. Investigation was undertaken to identify if there was any link between the cases. A further fatal case (Case 4) in an adult was reported to the Health Protection Unit on 5 January 2010. This case had died abroad but had travelled during the seven-day incubation period from the same town as the other fatal cases.

The cluster was investigated for any epidemiological links that might indicate local transmission pathways. Clinical information and the movements of the cases in the seven days before their onset of illness were gathered to determine any spatio-temporal links. We calculated the three-month (October–December 2009) incidence per 100,000 population in the town for children in the 0–4-years age group (as the three initial cases were all below four years of age) to help determine whether an outbreak (more cases than expected) was occurring. Overall incidence and subtype-specific incidence were calculated separately. Only the first case in any household was used in the calculation of local incidences. The local authority boundary of the town was used as the population boundary for the incidence calculations. The UK guidelines on meningococcal disease discuss the criteria for implementing community-level interventions in a defined population, when an age-specific incidence exceeds 40 per 100,000 population [1].

Microbiological investigations

No ante-mortem clinical samples were available for Case 1; however, a post-mortem blood sample was taken for polymerase chain reaction (PCR) investigation (carried out at Great Ormond Street Hospital, London). DNA extracted from the blood sample was subsequently forwarded to Meningococcal Reference Unit for genosubtyping (*porA* gene sequencing). Genogrouping was carried out by TaqMan real-time *siaD* PCR assay following initial TaqMan real-time *ctrA* PCR assay screening [7].

Oropharyngeal samples for culture and peripheral blood samples for culture and PCR were taken from Cases 2 and 3. Results of the microbiological investigation of the fourth case were requested from the country where the case died.

Genosubtyping

The Meningococcal Reference Unit provided genosubtype information for three of the cluster cases. Retrospective genosubtyping of all the cases of serogroup B invasive meningococcal disease confirmed in Suffolk during 2009 was also carried out, to compare with those of the cluster and identify any predominant genosubtype in the community. There were a total of 19 cases of serogroup B invasive meningococcal disease in Suffolk in 2009, including the two cases from the cluster (for which the genosubtype was available). Therefore samples from a further 17 cases were investigated retrospectively by the Unit, seven of which were confirmed by PCR only (culture negative). Although all case isolates are routinely characterised by *porA* gene sequencing, for patients whose infection is confirmed by PCR alone, the *N. meningitidis* genosubtype is determined only in specific instances of public health interest, as the assay is difficult.

Results

Case investigation and microbiological investigation

The first fatal case (Case 1) notified on 14 December 2009 was appropriately managed as a single case of meningococcal disease according to national guidelines. The *N. meningitidis* serogroup for this case was not known in the early stages of investigation as clinical samples were not available; however PCR investigation of post-mortem samples later confirmed this as serogroup B. Case 2 was confirmed as being infected with *N. meningitidis* serogroup B by PCR.

The clinical presentation of Case 3, who had an underlying condition, was not clinically typical of invasive meningococcal disease. Blood cultures taken

ante-mortem grew Gram-negative cocci. The organism was initially thought to be *N. meningitidis* but was later identified as *Veillonella atypica*, which is a Gram-negative coccus and strict anaerobe. The hospital laboratory considered that this was probably a contaminant. Case 3's PCR result (from a blood sample) and other cultures were negative for *N. meningitidis*. It was concluded that Case 3 did not have invasive meningococcal disease and therefore was excluded from the cluster.

A laboratory in the country where the fourth case died kindly provided confirmation of the case's serogroup (serogroup B), which was determined by PCR (from a skin biopsy).

The close contacts of all four cases were identified and given prophylaxis according to the UK national guidelines.

Genosubtyping data

Table 1 shows that Cases 1 and 2 were indistinguishable by *porA* gene sequencing, but no epidemiological links could be established between them. For Case 4 (the adult fatal case), data were only available for the *porA* VR1, due to limited availability of clinical material (skin biopsy). However, this was sufficient to show that Case 4 was distinct and was not linked to Cases 1 and 2.

A total of 17 samples from serogroup B invasive meningococcal cases in Suffolk in 2009 were retrospectively analysed to determine their genosubtype. Data were available for 15 samples only, as two samples were not typeable. The genosubtype information on 17 serogroup B invasive meningococcal cases (two from the 2009 cluster and 15 from retrospective analysis) for 2009 in Suffolk is shown in Table 2, which lists the *porA* variable region (VR) data. This clearly established that the *porA* subtype VR1=19-1, VR2=15-11, VR3= 36 (present in Cases 1 and 2 of the cluster of fatal cases) was one of the more common genosubtypes circulating in the community. This specific *porA* subtype represented 18% (three of 17) of all typeable serogroup B invasive meningococcal isolates in Suffolk and was the fourth most common genosubtype in England and Wales in 2009 (unpublished 2010 data from the Meningococcal Reference Unit).

Table 1

Serogroup and genosubtypes of the cluster of fatal cases of invasive meningococcal disease in a town in Suffolk, United Kingdom, December 2009–January 2010 (n=3)[a]

Case number	Age or age range (years)	Serogroup	*porA* genosubtype
1	0–4	B	VR1=19-1, VR2=15-11, VR3=36
2	0–4	B	VR1=19-1, VR2=15-11, VR3=36
4[b]	Mid-40s	B	VR1=7

VR: variable region.

[a] Case 3 was excluded from the cluster as this case did not have invasive meningococcal disease.

[b] Only data on the *porA* VR1 were available for Case 4, due to limited availability of clinical material (skin biopsy).

Table 2

Data on *porA* genosubtypes of all typeable cases of serogroup B invasive meningococcal disease in Suffolk, United Kingdom, 2009 (n=17)[a]

Number of cases per genosubtype	*porA* genosubtype
5	VR1=22, VR2=14, VR3=36
4	VR1=22, VR2=9, VR3=35-1
3	VR1=19-1, VR2=15-11, VR3=36
1	VR1=22, VR2=14-26, VR3=36
1	VR1=21, VR2=16, VR3=37-1
1	VR1=7-2, VR2=4, VR3=37
1	VR1=5-1, VR2=10-1, VR3=36-2
1	VR1=7-1, VR2=1, VR3=35-1

VR: variable region.

[a] Of the 19 serogroup B invasive meningococcal cases in Suffolk in 2009, *porA* sequence subtype data were available for 17 cases.

The genosubtype determined for Cases 1 and 2 of the cluster of fatal cases in December 2009 to January 2010 is highlighted.

Epidemiological analysis

The three-month (October to December 2009) incidence of serogroup B invasive meningococcal disease in children aged 0–4 years in Suffolk was 37 per 100,000 population. The incidence of the disease caused by the *N. meningitidis* genosubtype VR1=19-1, VR2=15-11, VR3=36 in the same age group for the same period was 24.6 per 100,000 population. This *porA* genosubtype, identified for Cases 1 and 2 of the cluster of fatal cases, was one of the common *porA* subtypes among isolates from cases of serogroup B invasive meningococcal disease from England and Wales in 2009. Retrospective *porA* sequencing of DNA from all the available Suffolk 2009 case material confirmed one other patient with a *N. meningitidis porA* subtype indistinguishable to that of Cases 1 and 2 of the cluster; however, this was a non-fatal case reported in the summer of 2009, in another part of the county.

There were two other cases of serogroup B invasive meningococcal disease in late December 2009 in Suffolk (in different towns to the cluster of fatal cases): both had a common *porA* subtype but were not epidemiologically linked and the *porA* subtype was distinct from that of Cases 1 and 2 of the cluster.

Control measures

The main control measure was to identify close contacts, as recommended in the UK national guidelines, and arrange for appropriate chemoprophylaxis. The guidelines recommend that the antibacterial agents rifampicin, ciprofloxacin and ceftriaxone be used to prevent secondary cases. Locally, rifampicin was used for persons aged under two years and ciprofloxacin for persons aged over two years. For pregnant or breastfeeding women, rifampicin or ceftriaxone are used.

Rifampicin is the only antibiotic that is licensed in the UK for the purpose of chemoprophylaxis.

Local general practitioners and out-of-hours services including accident and emergency departments were advised to be on the alert for new cases of meningococcal disease. In addition, information on signs and symptoms of the disease, including advice to seek medical advice rapidly, was provided to the local community using the local media.

Discussion and conclusions

The identification of a community outbreak is a challenge, especially when considering community interventions where the population boundary will of necessity be arbitrary. The options for community interventions are limited in outbreaks of serogroup B invasive meningococcal disease [8]. Community interventions include antibiotic prophylaxis and for serogroups A, C, Y or W135, vaccination against the relevant outbreak organism. There are no licensed serogroup B vaccines currently available in the UK. Strain-specific outer membrane vesicle vaccines have been used in some countries such as New Zealand [9] and France [10] to control a national and regional epidemic of serogroup B meningococcal disease dominated by a single strain. Mass chemoprophylaxis has disadvantages: it can lead to potentially serious complications and development of resistance [11]. It could also result in the elimination of non-pathogenic *Neisseria* species, leading to reduced immunity against pathogenic species, and would not prevent the reintroduction of the outbreak strain after the prophylaxis [12,13].

The UK guidelines recommend intervention in community outbreaks if the age-specific incidence in a three-month period is high and a pragmatic threshold for triggering intervention has been set. Targeted community interventions have been implemented in the UK in the past, where the age-specific incidence exceeded 40 per 100,000 population in serogroup C outbreaks of four or more cases [1].

The Communicable Diseases Network Australia guidelines define a community outbreak as three or more confirmed cases of a specific serosubtype in a three-month period with an incidence rate of 10 per 100,000 population and explicitly states that mass chemoprophylaxis should not be used in community outbreaks [14]. Australia, like the UK, currently has no licensed serogroup B vaccines.

Similarly the US CDC guidelines on prevention and control of meningococcal disease state that currently there is no licensed serogroup B vaccine available in the US and mass chemoprophylaxis is not generally recommended for large outbreaks in the community due to the disadvantages of chemoprophylaxis [12]. The CDC recommends that in outbreaks involving limited populations (e.g. an outbreak in a single school), administration of chemoprophylaxis could be considered in serogroup B outbreaks [15].

The guidelines on community interventions for outbreaks of serogroup B are similar across countries and the options are fairly limited, as discussed above. Nevertheless, it is important to identify a community outbreak or rule out an outbreak in order to implement appropriate public health interventions. Meningococcal disease clusters can cause a great deal of public anxiety and media interest, especially when they involve fatal cases. In our cluster investigation, we found the incidence of disease caused by the *N. meningitidis* genosubtype VR1=19-1, VR2=15-11, VR3=36 (24.6 per 100,000 population) for children aged 0–4 years was well below the trigger for any community interventions according to UK guidelines. It is important to note that the local authority boundary was chosen as an arbitrary geographical boundary (population boundary) for the incidence calculations.

Although epidemiological links are useful in identifying transmission pathways in clusters and outbreaks, molecular techniques to characterise the isolates provide extremely useful supporting information [16, 17]. In our investigation we found that genosubtyping (using *porA* gene sequencing) of the N. Meningitides, infecting cases in the town and the data on the incidence of the various genosubtypes of serogroup B invasive meningococcal disease in England and Wales were useful in delivering an appropriate public health response. Although the two fatal cases in December 2009 were of indistinguishable genosubtype, this subtype was found to be one of the common strains and it was concluded that they were likely to be sporadic cases. Testing for additional antigens or other genes for variation (discrimination) was not routinely carried out at the Meningococcal Reference Unit. At the Unit, given the practicability and discrimination afforded by *porA* sequencing, developed for culture and non-culture material, this approach has been used for the investigation of clusters. Multilocus sequence typing (MLST) is currently not used routinely for the investigation of clusters, but has recently been agreed by the Health Protection Agency as the preferred second-line discrimination assay. Testing for other gene targets (such as ferric enterochelin receptor, fetA, and factor H binding protein, fHBP) are under investigation but the practicality and economic feasibility of such tests remain to be determined.

It was determined that two non-fatal invasive meningococcal cases in Suffolk between 25 and 31 December 2009 had an indistinguishable *porA* gene sequence (VR1=22, VR2=9, VR3=35-1), but it was different to that of Cases 1 and 2. These non-fatal cases were from different parts of the county, with no epidemiological links. They were sporadic cases caused by one of the other common strains in the UK. The *porA* gene sequencing data on all typeable 2009 Suffolk invasive meningococcal cases obtained from the Meningococcal

Reference Unit proved very useful in understanding the dynamic nature of different strains circulating in Suffolk. In the investigation of clusters, *porA* gene sequencing can be performed to differentiate between strains within the timescale required for public health action [14]. Sequencing of the *porA* gene was a very useful tool in the risk assessment of our investigation.

In conclusion, extensive investigation failed to establish an epidemiological link among the fatal cases of serogroup B invasive meningococcal disease in this reported cluster. The genosubtyping of the isolates proved important in the public health management of this incident by showing that the cases were not part of an outbreak with transmission links, in which mass chemoprophylaxis might have been considered.

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Pandemic influenza A(H1N1)2009: molecular characterisation and duration of viral shedding in intensive care patients in Bordeaux, south-west France, May 2009 to January 2010

L Malato[1,2], V Llavador[1,2], E Marmier[1], J Youssef[3], C Balick Weber[3], H Rozé[3], E Bessede[4], H J Fleury (herve.fleury@viro.u-bordeaux2.fr)[1]
1. Virology laboratory, University Hospital of Bordeaux, France
2. These authors contributed equally to this work
3. Intensive care units (Hospitals St André, Pellegrin and Haut Lévèque), University Hospital of Bordeaux, France
4. Bacteriology Laboratory, University Hospital of Bordeaux, France

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From May 2009 to January 2010, the Virology Laboratory at the University Hospital of Bordeaux received more than 4,000 nasopharyngeal samples from the Aquitaine region (south-west France) for the diagnosis of pandemic influenza A(H1N1)2009. Eighty-three infected patients deteriorated and were admitted to intensive care units. Our study focused on 24 of these patients. Positivity for influenza A(H1N1)2009 was monitored by realtime PCR and duration of viral shedding was determined. The first available sample of each patient was analysed for bacterial, fungal and viral co-infection. We observed six bacterial (or bacterial/fungal) co-infections and one viral co-infection with respiratory syncytial virus. The samples were analysed for the presence of the neuraminidase H275Y (N1 numbering) mutation, which confers resistance to oseltamivir, by realtime PCR of the neuraminidase gene. No H275Y mutation was observed in any of the viral strains screened in this study. In parallel, a fragment of the haemagglutinin gene encoding amino acid residues 173 to 362 was sequenced to detect mutations that had been reported to increase the severity of the disease. Two patients were infected by strains bearing the D222G (H3 numbering) mutation. The viral shedding of A(H1N1)2009 in this study ranged from four to 28 days with a median of 11 days.

Introduction

During the influenza A(H1N1)2009 pandemic, the virology laboratory at the University Hospital of Bordeaux received from May 2009 to January 2010 more than 4,000 samples collected from the Aquitaine region (south-west France), an area with three million inhabitants. Some 1002 (24.9%) samples were confirmed as positive for pandemic influenza A(H1N1)2009 by real-time PCR. During this period, the three intensive care units (ICUs) of the University Hospital of Bordeaux received 83 patients with severe clinical conditions including acute respiratory distress syndrome (ARDS). Six of them required extracorporeal membrane oxygenation (ECMO) support. We could study those six and an additional 18 influenza-positive ICU patients in detail to address the following points: to establish the presence of microbial co-infection on admission, to obtain molecular data on the oseltamivir resistance-associated H275Y mutation [1] in the neuraminidase gene, to screen for already identified mutations in the haemagglutinin (HA) gene that may have an influence on the virulence of the virus [2-5], and to evaluate the duration of viral shedding.

Methods

Patients with confirmed influenza A(H1N1)2009 were selected retrospectively for this study after their admission to the ICU for influenza complications, for example respiratory failure or exacerbation of an underlying chronic condition requiring surveillance or assistance. The patients in this study were admitted to the ICU between May 2009 and January 2010.

The detection of influenza A(H1N1)2009 viral RNA was carried out in nasal swabs, bronchoalveolar lavage fluids or respiratory secretions. Pandemic influenza A(H1N1)2009 was diagnosed using the Roche detection kit for influenza A (RealTime ready Influenza A(H1N1) detection set) and operated on a Roche LightCycler 480.

We screened each patient at admission for viral, bacterial and fungal co-infections. Viral respiratory co-infections were investigated using a multiplex PCR assay (Seegene Seeplex RV5-ACE screening) which allows the detection of influenza A, influenza B, respiratory syncytial virus (RSV) A/B, adenovirus A/B/C/D/E, parainfluenzavirus 1/2/3, bocavirus 1, metapneumovirus, human rhinovirus and coronavirus OC43/229E/NL63/HKU1. Bacterial and fungal co-infections were diagnosed after culture and/or serology.

The H275Y (N1 numbering) mutation conferring resistance to oseltamivir was investigated on admission on the first specimen by a fluorescence resonance energy transfer (FRET)-based assay designed in the virology laboratory in Bordeaux as previously described [6].

For sequencing of the HA gene, influenza A RNA was reverse-transcribed using the Titan One Tube RT-PCR kit (Roche) with primers HA1S (ATGAAGGCAATACTAGTAGTTATGCTATATAC) and HA1AS (TTAAATACATATTCTACACTGTAGAGACCC). cDNA was then subjected to a nested PCR to amplify a fragment encoding for amino acid residues 173-362 with primers HA3S (CCAAAGCTCAGCAAATCCTAC) and HA3AS (ATCTCGTCAATGGCATTCTGT). The sequences were aligned to the reference strain A/California/06/2009 using Clustalw and Jalview softwares.

Duration of viral shedding was determined as the period between the onset of symptoms and the last positive PCR for influenza A(H1N1)2009 with exception of some cases for whom onset of symptoms could not be determined (the first positive PCR being used as Do of viral shedding). As there was no standard protocol for the follow-up of influenza patients, sampling could have stopped while the patients were still positive for influenza A(H1N1)2009. Using such a method we may have underestimated the duration of the shedding but were not dependent on a negative PCR to evaluate the shedding.

Results

We studied 24 patients admitted to the ICU for severe influenza A(H1N1)2009 between May 2009 and January 2010. All the data collected are summarised in Table 1. The patients had a median age of 51.5 years ranging from 2 to 85 years and the female:male sex ratio was 0.45. Eight patients were immunocompromised (one with lung carcinoma with metastasis, one with co-infection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), two with leukaemia, two with lymphoma and two patients under follow-up for transplantation), seven had chronic cardiovascular and/or pulmonary diseases, four were obese (BMI>30), and nine had no comorbidity. During the study four patients died.

We were able to collect data concerning antiviral treatment for 20 of the 24 patients. The 20 patients had received the neuraminidase inhibitor oseltamivir. The median time of oseltamivir treatment initiation in the 17 patients for whom this information was available, was five days after the onset of symptoms (range: 1-12 days).

Screening on admission for microbial co-infections revealed only one viral co-infection with respiratory syncytial virus (RSV) and six bacterial or fungal co-infections: *Staphylococcus aureus, Haemophilus influenzae, Streptococcus agalactiae, Branhamella catarrhalis, Enterobacter cloacae, Mycoplasma pneumoniae* and *Candida albicans* (Table 1)

We were able to follow up positivity for influenza A(H1N1)2009 viral RNA in 18 patients for whom we had several specimens. The median duration of viral shedding was 11 days (4-28 days, Table 2). Immunodepression was associated with prolonged viral shedding, with six of the eight immunocompromised patients PCR-positive 14 or more days after onset of symptoms (Table 1); the two other patients who also shed virus for longer than 14 days were obese. Immunocompetent and immunocompromised patients shed virus for a median duration 10 days and 16 days, respectively.

The H275Y mutation was not detected in any of our patients, nor was any other mutation at position 275 of the neuraminidase gene.

We amplified 26 HA sequences from 21 patients (two patients were investigated with several successive samples). The different substitutions of our isolates compared to the reference strain are shown in the Figure. Three samples from two different patients exhibited the D222G substitution. The first (Patient 1 in Table 1) was a patient with morbid obesity (body mass index>40) presenting a severe ARDS requiring ECMO support for nine days and mechanical ventilation for a further 20 days. The HA sequence of virus isolated from their bronchoalveolar lavage fluid showed a mixed population at codon 222: D222EG. As shown in Table 1, she exhibited prolonged viral shedding of 28 days (already published [7]) but recovered and was discharged after one month. The second case (Patient 8 in Table 1) had a lymphoma and chronic obstructive pulmonary disease. Viral shedding lasted for a minimum of 14 days (from the first to the last positive sample), and the patient died after 19 days of hospitalisation. Four influenza A-positive samples from this patient were subjected to HA sequencing. The first sample, a nasal swab, did not contain the D222G substitution, nor did the second one which was a respiratory secretion. Interestingly, the D222G was identified in the third and fourth specimens obtained from secretions 12 and 14 days after the first sample. A mixed population (D222DG) was noted in the fourth specimen. In addition to the D222G mutation, isolates from all four samples contained a V321F substitution in HA that did not match any HA sequences published as of May 2010.

Other substitutions are listed in Table 3 and include S203T (13/26 sequences), and less frequently D222E (4/26), Y230H (1/26), M257I (1/26), Q293H (1/26), I295V (2/26), K305R (1/26), V321I (2/26) and V321F (5/26).

Discussion

In Aquitaine, 13–25% of the population were infected with influenza A(H1N1)2009 during the pandemic [8].. Between May 2009 and January 2010, 83 patients suffered from a complicated influenza and were admitted

Table 1

Clinical and microbiological features of influenza A(H1N1)2009 patients requiring intensive care, Bordeaux, May 2009- January 2010 (n=24)

Patient	Age group (years)	Sex	ECMO	Outcome	Immunodepression	Respiratory symptom	Cardiac symptom	Obesity[a]	Viral co-infection	Bacterial or fungal co-infection	Median time of NAI treatment initiation (days)	Viral shedding days)	D222G in HA
1	15-44	F	Yes					Yes			5	28	Yes
2	45-64	M	Yes		Hairy cell leukaemia			Yes			7	27	
3	45-64	F	Yes	Deceased	Cardiac transplantation						1	19	
4	45-64	M	Yes		Chronic lymphocytic leukaemia						ND	17	
5	15-44	M			HIV/HCV					*Haemophilus influenzae/ Candida albicans*	8	16	
6	45-64	F	Yes					Yes			6	14	
7	45-64	M		Deceased	Lung cancer	Asthma					9	14	
8	45-64	M		Deceased	Lymphoma	COPD					5	14	Yes
9	45-64	M									12	12	
10	15-44	M								*Streptococcus agalactiae*	4	10	
11	45-64	M				Respiratory failure					ND	10	
12	45-64	M		Deceased						*Staphylococcus aureus*	8	10	
13	45-64	F	Yes			Asthma		Yes			8	9	
14	45-64	F				COPD					9	7	
15	45-64	M			Lymphoma						ND	7	
16	0-15	F								*Enterobacter cloacae/Mycoplasma pneumoniae*	3	4	
17	45-64	F					Cardiopathy				ND	4	
18	15-44	M									4	4	
19	45-64	F							RSV	*Candida albicans*	2	ND	
20	15-44	F									ND	ND	
21	65+	F									ND	ND	
22	65+	F				Chronic bronchitis					ND	ND	
23	0-15	M								*Branhamella catarrhalis*	1	ND	
24	15-44	M			Lung transplantation						1	ND	

COPD: Chronic obstructive pulmonary disease; ECMO: extracorporeal membrane oxygenation; F: female; HA: haemagglutinin; HCV: hepatitis C virus; HIV: human immunodeficiency virus; M: male; NAI: neuraminidase inhibitor; ND: not determined; RSV: respiratory syncytial virus.

[a] Obesity was defined as a body mass index>30.

to an ICU. Influenza A(H1N1)2009 has been widely reported to affect subjects younger than those usually affected by seasonal influenza, i.e. people over than 65 years of age or with underlying chronic conditions [9,10]. This is in accordance with the 24 severely ill patients in our study who had a median age of 51 years. While one third of them (9/24) did not have an identified risk factor for influenza, the remaining two thirds were either immunocompromised or presented with underlying respiratory and/or cardiac disease or were obese.

While ARDS was also observed in previous seasonal influenzas, it was more frequent with the pandemic influenza A(H1N1)2009 virus strain. ARDS was responsible for 36–96% of admissions to ICUs during the pandemic [11-14] and might account for the increased need for ECMO support [15]. Among the 83 critically ill patients in Aquitaine, ARDS was involved in 57 [8]. Seven of the 83 received ECMO support for a median duration of 16 days, and six of those were included in the present study. Patients under ECMO in this study were either immunocompromised and/or obese. This is in line with other studies that identified obesity as a comorbidity for influenza A(H1N1)2009, as already noted in previous studies [13,16,17].

Our patients were screened on admission to the ICU for microbial co-infections that could increase the severity of the influenza. Viral co-infection was scarce: one case of influenza/RSV co-infection was seen in a patient in their 60s with no risk factors. In addition, we found six bacterial/fungal co-infections, the majority of which were not acquired in the hospital. While one of them, Patient 12, died of fatal septicaemia caused by a *S. aureus* infection, there was no suspicion that the bacterial co-infection had an effect on the severity of influenza in the five remaining patients. A recent study reported that the role of bacterial co-infection in the need for ICU admission is not clear, but that the virus is the cause of critical illness in the vast majority of cases [18].

The median duration of viral shedding was 11 days in our study, which is longer than the five to seven days noted in uncomplicated A(H1N1)2009 cases [19-21]. Eight of the nine patients shedding virus particles for longer than the median had an identified risk factor, among others immunodepression and/or obesity that are considered as a poor prognostic factor. However, the ninth patient had no comorbidity. It is widely accepted that the period of shedding of influenza virus is longer in immunocompromised patients [19,20]. Interestingly, viral shedding was longer in patient 1 (with a 28-day peak) whose immunological status was normal but who was obese. Furthermore, patients with ECMO or with a fatal outcome had longer viral shedding values than others.

The patients included in this study had been treated with the neuraminidase inhibitor oseltamivir. The

Table 2
Viral shedding in intensive care patients determined by detection of A(H1N1)2009 RNA by realtime PCR, Bordeaux, May 2009–January 2010 (n=18)

Patient	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
1					+	+		+		+	+	+	+	+		+			+						+	+	+		
2								+					+														+	+	
3	+			+		+				+					+			+	+					+				
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8	+		+			+	+			+		+		+															
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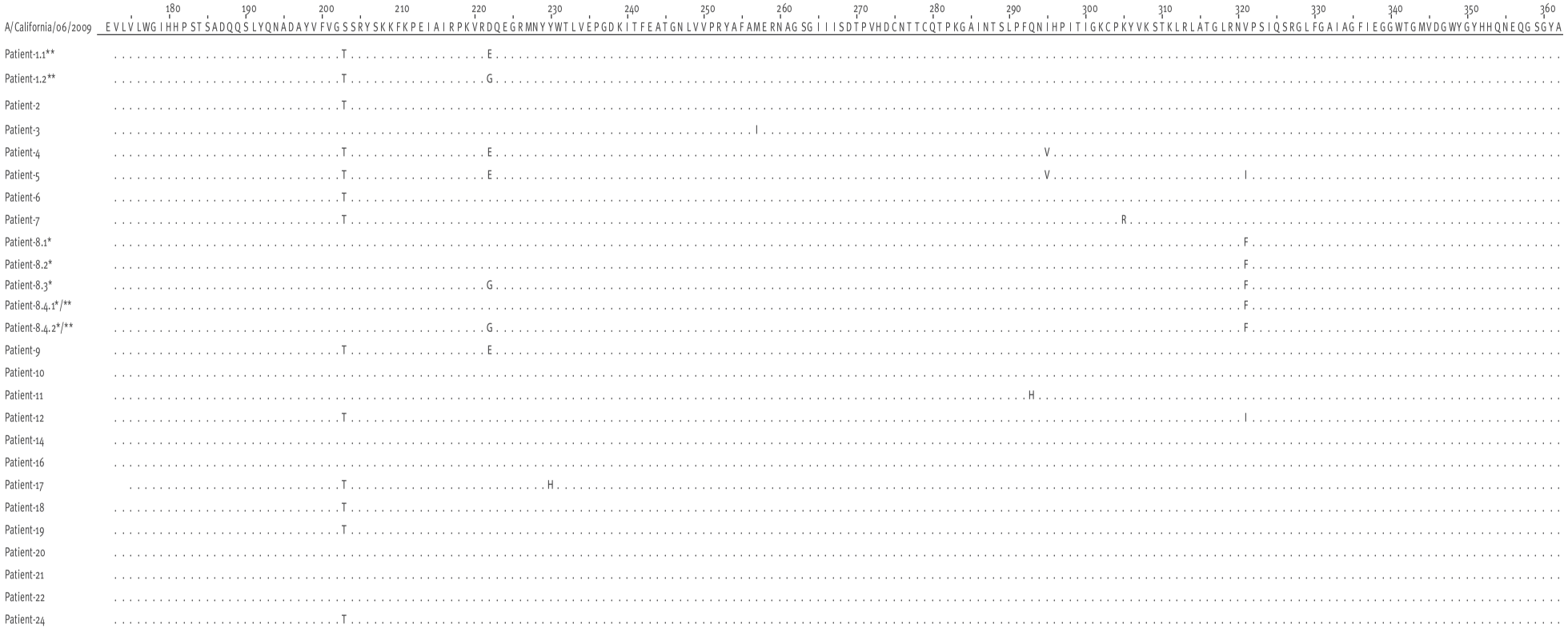
15	+						+																						
16	+			+																									
17	+			+																									
18				+																									

Day: Day of onset of symptoms (or first positive PCR when onset of symptoms could not be determined).

Figure

Alignment of influenza A(H1N1)2009 haemagglutinin amino acids 173-362 from intensive care patients with reference strain A/California/06/2009, Bordeaux, May 2009- January 2010 (n=21 patients)



* Four specimens were available for Patient 8.

** Sequences exhibiting a mixed population of substitutions were considered and processed as two different readings.

Only substitutions are shown. Seven residues were polymorphic.

median delay before initiation of treatment was five days, which exceeds the recommended time for the administration of oseltamivir at the latest 48 hours after the onset of symptoms [22]. Late treatment due to delayed admission to the ICU and comorbidities could account for prolonged viral shedding because of a slower viral clearance [23]; it has been shown that treatment initiated one to three days after infection significantly shortens viral shedding duration [24]. However, Patient 3 was shedding virus particles for 19 days despite rapid administration of oseltamivir.

As among the currently licensed drugs only neuraminidase inhibitors remain useful to treat influenza A(H1N1)2009, it is of particular importance to monitor the resistance/sensitivity of viral isolates to oseltamivir. Unfortunately worrying levels of oseltamivir-resistant isolates of the seasonal influenza A(H1N1) have emerged in Europe [25,26]. In these viruses, the most frequent mutation conferring resistance to oseltamivir is the H275Y substitution [27] in the neuraminidase gene, which does not cause cross-resistance to zanamivir.

Among the 26 isolates analysed, we have not observed any H275Y substitution. These data are in accordance with the literature showing that the prevalence of resistant A(H1N1)2009 viruses is at present very low. As of August 2010, 304 cases of oseltamivir resistance in this strain have been reported worldwide [28], all of which were due to the H275Y mutation in NA.

The HA protein is one of the determinants of virulence and host specificity through it's interaction with the sialic acid receptor on the cell surface. While avian influenza viruses preferentially bind to alpha2,3–linked sialic acid, human viruses prefer the alpha2,6 linkage [29]. It has been shown that two positions in HA are involved in determining sialic acid binding preference, namely amino acid residues 187 and 222 (190 and 225 in H3 numbering) [30]. A D222G mutation causes a shift to preferential binding to alpha2,3 receptors. This mutation has recently been described in influenza A(H1N1)2009 isolates from patients with severe disease or fatal outcome in several countries [2,4,5,31,32], but has also been detected in association with a mild disease [33].

Two D222G substitutions were observed in our study. Both patients experienced a severe clinical course of disease. One required ECMO and the estimated viral shedding lasted 28 days [7], while the other died after 19 days and was at the time probably still positive for influenza A(H1N1)2009, although no autopsy was performed. In the deceased patient, this mutation was not present on admission but appeared 12 days after the first positive sample, therefore suggesting a selection event. We propose that the long duration of viral shedding allowed the virus to evolve and acquire this substitution. Whether or not this mutation accounted for the severity of the disease in this patient remains to be investigated.

Interestingly, the 1918 Spanish influenza isolate NY18 carried the combination D190/G225 and had double specificity for both alpha2,3– and alpha2,6–linked sialic acid [30]. It has been shown in ferrets that this viral isolate fails to transmit efficiently but remains virulent [30,34]. Alpha2,3 sialic acid receptors are found in the lower respiratory tract in humans [35]. Like the avian influenza A(H5N1) virus, strains with mutations that affect receptor binding might be less efficiently transmitted but could have an increased pathogenicity [4].

In addition to the D222G substitution, we observed four D222E substitutions in this study (Table 3, Figure). Although these patients had prolonged viral shedding, we could not clearly establish a link with the severity of the disease as they all, except Patient 9, presented comorbidities. Studies have shown that the proportion of D222E is similar in mild and severe cases [32].

In parallel, we found Q293H and I295V mutations whose pejorative role has been mooted but remains to be confirmed [3].

Table 3

Frequency of haemagglutinin substitutions identified in influenza A(H1N1)2009 isolates from intensive care patients, Bordeaux, May 2009- January 2010 (n=21 patients)

Mutations in HA	Frequency (among the 26 sequences)	Number of patients exhibiting this mutation
S203T	50%	12
D222G	8%	2
D222E	15%	4
Y230H	4%	1
M257I	4%	1
Q293H	4%	1
I295V	8%	2
K305R	4%	1
V321I	8%	2
V321F	19%	1

HA: haemagglutinin.

Conclusion

In 24 patients hospitalised in the ICU for pandemic influenza A(H1N1)2009 infection, the requirement for ECMO was mainly associated with comorbidities (immunodepression/pulmonary disease/obesity) and long viral shedding despite oseltamivir treatment.

All strains were found susceptible to oseltamivir. The D222G substitution was observed in only two patients and we hypothesise that this mutation is selected for in the lower respiratory tract but is not transmitted. Microbial co-infections were detected, but with one exception it was not clear whether they contributed to the severity of the disease. We think that the influenza virus alone was responsible for the severe disease and the evolution toward ARDS.

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Letter to the editor. Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in winter 2010/11

F Baldanti (f.baldanti@smatteo.pv.it)[1], A Zanetti[2]
1. Molecular Virology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
2. Department of Public Health, Microbiology and Virology, University of Milan, Milan, Italy

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To the editor: In response to the paper by Ellis *et al.* [1], published in Eurosurveillance Volume 16, issue 1, we would like to make a few observations.

D222G/N substitutions in the haemagglutinin of influenza A(H1N1)2009 strains have been associated with increased virulence [2-4].

An enhanced binding affinity of the mutated haemagglutinin to the α-2,3 sialic acid receptor rather than to the α-2,6 sialic acid receptor has been postulated to be the basis of the increased virulence of D222G/N mutants [3]. The α-2,3 sialic acid receptor is present at higher density on the surface of the cells of the lower respiratory tract tissues whereas the α-2,6 sialic acid receptor is present at higher density on the surface of cells of the upper respiratory tract tissues.

In surveillance reports from different countries, the overall presence of such mutants ranged from 2.0% to 5.6%, while it was significantly higher (up to 22.9%) in severe or in fatal cases [2-4]. Interestingly, the paper by Kilander *et al.*, analysing both nasal swabs and bronchoalveolar lavage, showed the highest rate of D222G/N mutants in patients with severe and fatal infections [2]. In a multicenter study, we analysed paired nasal swabs and bronchoalveolar lavage samples from patients admitted to intensive care units for mechanical ventilation or extracorporeal membrane oxygenation. The samples were compared with samples from patients with pneumonia not requiring mechanical ventilation and from community patients. Our data showed that D222G/N mutants were more frequently detected in lower respiratory tract secretions than in secretions from the higher respiratory tract [5]. In addition, by combining data from nasal swabs and bronchoalveolar lavage samples, the frequency of D222G/N mutants in patients with severe infections increased to 43.0%, as compared to 7.8% and 0% in patients with moderate and mild infections, respectively [5]. In agreement with the pathogenetic hypothesis considering the lower respiratory tract as the more favorable environment for replication of such mutants, viral RNA levels were significantly higher in bronchoalveolar lavage samples than in nasal swabs [5].

Ellis *et al.* reported that almost all viruses derived from fatal and non-fatal cases analysed (39/41) in the United Kingdom during the early wave of the 2010/11 influenza winter season showed the wild-type 222D haemagglutinin residue [1]. Thus, in the paper by Ellis *et al.*, severe and fatal influenza cases were not associated with the emergence of D222G/N mutants, even though the role of other aminoacid substitutions remains to be determined [1]. The authors of this study do not specify the type of clinical samples used for analysis. In the case they used nasal swabs only, on the basis of the above-referenced studies, the rate of D222G/N mutants might have been underestimated.

For a better understanding of the mechanisms of influenza A pathogenicity and the epidemiology of severe and fatal events, the analysis of bronchoalveolar lavage specimens in parallel with nasal swab specimens from patients admitted to intensive care units for severe infections should be envisaged. Additionally, a post-mortem analysis of tissues and/or secretions from the lower respiratory tract from deceased patients, in the event they had not been previously analysed, is important.

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Authors' reply. Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in winter 2010/11

J Ellis (joanna.ellis@hpa.org.uk)[1], M Galiano[1], R Pebody[1], A Lackenby[1], CI Thompson[1], A Bermingham[1], E McLean[1], H Zhao[1], S Bolotin[1], O Dar[1], J M Watson[1], M Zambon[1]
1. Health Protection Agency, Centre for Infections, London, United Kingdom

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To the editors: Our Italian colleagues provide commentary on an important question, as yet unresolved, regarding the relationship between pathogenesis of influenza A(H1N1)2009 infection and mutation in particular viral genes contributing to virulence. Viral haemagglutinin (HA) is the key virulence determining gene for influenza in birds, and a major determinant for host cell tropism in mammalian influenza [1]. The link between cell tropism and virulence in humans remains unclear; many different approaches to this question conclude that virulence is associated with multiple viral genes, including genes determining replication efficiency (polymerase genes) and non-structural genes governing the interaction with the host immune response.

The emergence of animal viruses into the human population is associated with adaptive mutations [2-3] and tracking substitutions at residues known to be associated with such adaptive changes is an important surveillance function. The commentary highlights the opportunities arising from surveillance to develop and apply hypothesis generating questions from observational data sets.

During the 2009 pandemic, attention has focussed on amino acid substitutions at position 222 in the HA of influenza A(H1N1)2009 viruses, which has been observed to vary [4], with aspartic acid (D), glutamic acid (E), asparagine (N) and glycine (G) residues being present at this position. There is a clear correlation between enhanced binding to α2-3-linked sialyl receptor sequences by 222G variants and increased infection of ciliated epithelial cells in vitro models [5].

Our rapid communication of data obtained during the early phase of the epidemic in winter 2010 in the United Kingdom, using available material predominantly derived from swabs taken at the point of diagnosis from the upper respiratory tract (URT), was intended to provide a comprehensive update from all available sources, to give as full a picture as possible. We agree that wherever possible, when URT and lower respiratory tract (LRT) samples are available from individual cases, analysis in parallel is important, as well as sequential sampling from individuals who are hospitalised with severe illness. The ability to link both of these observations to clinical outcome and "within host" variation or evolution is important. We recognise that there is an inherent bias in such an approach, as individuals in the community are almost never sampled from the LRT, leading to the possibility of over interpretation of the importance of a single mutation, by focussing only on severe cases, but analysis of clinical outcome and its relationship to whole genome genetic composition of influenza viruses is underway in several different centres internationally.

The selection and emergence of the D222G mutation as a cause or consequence of more severe lower respiratory tract infection is still to be resolved. Emergence of this mutant is likely to exacerbate severity of disease, but by itself, may be neither necessary nor sufficient to account for a severe disease outcome, which is invariably a balance between virus virulence factors and host immune response capability. Further work is needed, both at the level of reductionist experimental pathology work in the animal model, and at the observational level in human populations. Detailed studies such as the Mechanisms of Severe Acute Influenza Consortium (MOSAIC) study [6], which focus on analysis of viral virulence and host immune response in severe illness, are likely to provide insights useful to understanding pathogenesis in humans. We thank our colleagues for raising this comment and for the opportunity to broaden the commentary in more detail than was possible in the original article.

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Call for applications for EPIET fellows

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

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