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Accepted for the impact factor – what is the impact of Eurosurveillance?

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Publications in impact factor journals have become an essential determinant of scientific careers and their listing plays a central role in the evaluation of grants, promotions etc. The scientific community judges the quality and importance of a journal and of individual publications by the impact factor which is awarded by a company called Thompson Reuters.

After several recent improvements to the journal, such as the merging of the former monthly and weekly editions into one weekly edition in January 2008 and the launch of a new website in April 2008, we embarked on an evaluation, in collaboration with a medical librarian, of whether our journal met the inclusion criteria for an impact factor [1]. We applied for the impact factor in October 2008 and are pleased to announce that Eurosurveillance has recently been selected for coverage by Thomson Reuters and is now indexed and abstracted in the Science Citation Index Expanded (also known as SciSearch®) and in the Journal Citation Reports/ Science Edition, beginning with Volume 14(1) 2009.

The basis for the calculation of the impact factor is the frequency with which the average article in a given journal has been cited in a defined period [2]. For Eurosurveillance, we expect the allocation of our first official impact factor for 2011, after the two-year evaluation period. It will be calculated as a ratio with the total number of citable articles published in Eurosurveillance in 2009 and 2010 as the denominator and the number of citations these articles receive in indexed journals in 2011 as the numerator. Generally, citable items are articles, reviews, proceedings or notes, while editorials or letters to the editor are excluded.

Obvious challenges lie ahead of us, and we will maintain our efforts to select the most interesting articles of high quality for our readers while at the same time supporting capacity building across Europe by lending assistance to less experienced authors. However, in the field of public health and communicable diseases, the real impact of a journal is determined by more than the calculated value of the impact factor. When dealing with outbreaks or emerging diseases, it is important that authoritative information is disseminated rapidly and reaches a wide range of stakeholders. Public health experts and policy makers require scientifically sound information that will allow them to choose necessary and appropriate public health actions.

In the past months, Eurosurveillance has proven to have an impact on public health by documenting the emerging H1N1 influenza pandemic in numerous reports not only from Europe but also from North and South America, Asia, Australia and New Zealand. In 61 articles to date, we have covered relevant aspects of the pandemic from modelling and phylogenetic analysis to antiviral treatment and vaccination. The majority of articles were rapid communications that we were able to process within one week from submission thanks to authors and peer reviewers who agreed to work to tight deadlines despite their already high work load. We are grateful for this support, and the efforts have paid off. Papers published in Eurosurveillance were further disseminated through channels such as ProMEDMail (http://promedmail.oracle.com/pls/otn/f?p=2400:1000:) and the Lancet H1N1 flu resource centre (http://www.thelancet.com/H1N1-flu). They featured widely in the general media and caught the attention of many experts and high level policy makers. Articles in Eurosurveillance were cited by a co-chairman of President Obama's Council of Advisors on Science and Technology [3].

We are confident that authors, reviewers and readers will continue to support us in our efforts to publish relevant and influential information of high quality, and that in two years time, these efforts will be manifest not only in the assigned impact factor but also in our actual impact for public health in Europe.

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Enhanced surveillance of initial cases of pandemic H1N1 2009 influenza in Ireland, April - July 2009

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From 28 April to 18 July 2009 there were 156 cases of pandemic H1N1 2009 influenza confirmed in Ireland. During this time, Ireland was in containment phase, and detailed case-based epidemiological information was gathered on all cases presenting in the community and acute health care setting. Active case finding was performed among contacts of cases. Eighty percent of cases were in people under the age of 35 years, and 86% were imported. The most frequent symptoms were fever, sore throat, myalgia and dry cough. Nine people were hospitalised, no fatalities occurred.

Background

In late April 2009, a novel influenza virus led to human infection in Mexico. A public health emergency of international concern was declared by the World Health Organization (WHO) on 25 April 2009 [1]. Over the following weeks the virus spread rapidly to all regions of the world. Consequently WHO declared a phase 6 pandemic on 11 June 2009 due to evidence of community-level transmission in multiple countries globally [2].

In Ireland the National Pandemic Plan was implemented from 25 April; existing surveillance systems were augmented and pandemic H1N1 2009 influenza and enhanced surveillance commenced. On 28 April 2009, the first case of pandemic H1N1 2009 influenza was confirmed in Ireland.

Prior to April 2009, a number of surveillance systems were in place in Ireland to monitor influenza and clusters of influenza-like illness (ILI). These systems included year round surveillance by sentinel general practitioners (GPs), virological surveillance (sentinel and non-sentinel), hospital sentinel surveillance and statutory reporting of outbreaks of ILI and influenza under the Infectious Diseases Regulations [3].

Baseline seasonal ILI rate thresholds were set for the Irish population in 2008 based on surveillance of ILI between 2001 and 2008 [4]. New systems implemented in April 2009 included:

- enhanced case-based reporting of all cases of pandemic H1N1 2009 influenza using the national electronic reporting system, (Computerised Infectious Diseases Reporting system, CIDR);
- increased virological surveillance by the GP sentinel influenza surveillance scheme (number of samples to be taken by GPs increased from two to five per week);
- recruitment of additional sentinel GPs;
- expanded hospital sentinel surveillance;
- augmented mortality surveillance to identify excess all-cause deaths, excess pneumonia and influenza deaths; and
- surveillance of influenza-related calls to out-of-hours GP services.

We report on the enhanced case based surveillance of the first 156 confirmed cases of pandemic H1N1 2009 influenza up to 18 July 2009, when the strategy changed from containment to mitigation, and detailed case based surveillance of all cases ceased.

Methods

GPs and hospital clinicians reported all suspect cases of pandemic H1N1 2009 influenza to local departments of public health who in turn contacted and interviewed them. Public health staff completed case-based enhanced surveillance forms with information from these interviews. In order to facilitate active case finding for enhanced surveillance, the European Union case definition of 30 April 2009 was adopted [5]. As evidence emerged internationally in individual countries that they were experiencing community transmission (either by reporting of large numbers of cases, or by the country itself stating that community transmission was occurring), they were added to the list of countries where a travel history would be relevant for the clinical assessment. Staff from departments of public health contacted all persons who fit the criteria of the EU case definition for a case under investigation. They had a swab (nose and throat) that was submitted to the National Virus Reference Laboratory (NVRL) for testing. Samples from all cases under investigation for pandemic H1N1 2009 virus tested at the NRVL were confirmed with reverse-transcript PCR (RT-PCR).

Contact tracing of cases was undertaken and some additional cases were identified through this mechanism. Health authorities collated information on any clusters/outbreaks identified including the number of people involved and the type of outbreak. An outbreak of ILI was defined as three or more cases of ILI arising within a 72 hour period which met the case definition above and where an epidemiological link was established.

Enhanced surveillance data and laboratory results were entered into the CIDR to allow real-time exchange of information between the NVRL, regional departments of public health and the Health Protection Surveillance Centre (HPSC).

HPSC analysed the enhanced surveillance data to describe pandemic H1N1 2009 influenza in terms of age, sex, pre-existing

medical conditions of infected cases, presenting features and complications associated with the infection, as well as source, timing and clusters/outbreaks of disease.

Results

During the period 28 April to 18 July 2009, 156 confirmed cases of pandemic H1N1 2009 influenza were reported; 80 female (50.9%) and 76 male (49.1%). The median age of cases was 25.0 years (range: 0-73 years). Eighty percent of cases were in people under 35 years of age. Table 1 shows the number of confirmed cases by sex, five-year age group and age-specific incidence rate per 100,000 population.

After the first case of pandemic H1N1 2009 influenza on 28 April 2009, sporadic cases occurred until the middle of June, after which case numbers began to increase, with more than six new cases per day by early July (Figure). One hundred and thirty four (86%) cases were imported, 14 (9%) were infected in Ireland by an imported case and two (1%) were infected in Ireland without any identifiable travel association, information was missing for six (4%) cases.

Complete information on clinical symptoms was available for 106 (68%) cases (Table 2). For these, fever or history or fever (≥ 38º) was reported in 95%. Sore throat, dry cough, myalgia and headache were frequently reported symptoms. Most cases reported mild to moderate illness similar to seasonal influenza. Sixteen percent reported diarrhoea. Six cases (4%) were reported as having developed pneumonia due to pandemic H1N1 2009 influenza , all of whom recovered.

Nine people were hospitalised with pandemic H1N1 2009 influenza (hospitalisation rate 5%). Of these cases, four were children under 5 years of age, four were in the age group between five and 64 years and one aged 65 years. Data on pre-existing medical conditions and pregnancy was collected on all hospitalised cases. Two of the five adults had pre-existing medical conditions such as chronic respiratory disease, chronic heart disease, immunosuppression and diabetes mellitus. There were no pre-existing medical conditions reported in the paediatric cases. All hospitalised cases recovered, no fatalities occurred.

Twelve outbreaks of pandemic H1N1 2009 influenza were identified, involving a total of 38 people. One outbreak was in travelling companions while other outbreaks occurred within families and extended families. The number of people affected per outbreak ranged from two to six. All contacts of cases were offered chemoprophylaxis.

For three outbreaks information was available on attack rates which were 20%, 33% and 74% resepctively. Surveillance of influenza-like illness (ILI) and respiratory illness in general showed little change from from the baseline threshold for winter seasonal influenza activity.

GP sentinel surveillance over the eleven week period studied showed a small increase in ILI consultation rates, with a rate of 13.1 per 100,000 population being reported in the week ending 13 July, which was an increase in comparison to the rate of 8.8 per 100,000 population reported during the week ending 6 July. Six (4%) of cases of pandemic H1N1 2009 influenza were identified through this sentinel system. Sentinel hospital influenza surveillance found no increases in respiratory admissions up to 18 July. Analysis of all cause, and influenza- and pneumonia- related deaths showed no excess mortality compared with the same period in previous years and no outbreaks of non-pandemic influenza were notified up to 18 July.

Discussion

The epidemiology of the initial cases of pandemic H1N1 2009 influenza in Ireland was similar to that seen in other countries [6-13]. The majority of cases were children and adults under 35 years. Similar numbers of males and females were affected.

Table 1

Pandemic H1N1 2009 influenza cases by sex, age and age-specific incidence rates per 100,000 population, Ireland, 28 April - 18 July 2009 (n=156)

Age group [years]	Male cases (age-specific incidence rate)	Female cases (age-specific incidence rate)	Total cases (age-specific incidence rate)
0-4	11 (7.1)	6 (4.1)	17 (5.6)
5-9	3 (2)	3 (2.1)	6 (2.1)
10-14	2 (1.4)	3 (2.2)	5 (1.8)
15-19	9 (6.1)	11 (7.7)	20 (6.9)
20-24	15 (8.7)	14 (8.2)	29 (8.5)
25-29	7 (3.7)	16 (8.7)	23 (6.2)
30-34	5 (2.3)	5 (2.9)	10 (2.9)
35-39	6 (3.7)	5 (3.2)	11 (3.4)
40-44	6 (4)	3 (2)	9 (3)
45-49	2 (1.4)	3 (2.2)	5 (1.8)
50-54	2 (1.6)	4 (3.3)	6 (2.4)
55-59	5 (4.4)	2 (1.8)	7 (3.1)
60-64	1 (1.1)	2 (2.2)	3 (1.7)
65+	2 (1)	3 (1.2)	5 (1.1)
Total	**76 (3.6)**	**80 (3.8)**	**156 (3.7)**

The majority of infected experienced a mild self-limiting illness with fever, cough, sore throat and myalgia being the predominant symptoms. As with seasonal influenza, some people experienced more severe disease requiring hospitalisation. However, in contrast to seasonal influenza there was an under-representation of infection in older people.

The surveillance activities undertaken in the initial weeks of the pandemic had several strengths and weaknesses that should be borne in mind. The case definition adopted for pandemic H1N1 2009 influenza in the first few months of the pandemic was very specific with strict clinical and epidemiological criteria, particularly the epidemiological requirement to have travelled to an affected area, to have had contact with a confirmed case or to work in a laboratory testing cases. This was important when the numbers of cases were very small and anxiety in relation to the disease was very high, but it resulted in the vast majority of presentations for suspected pandemic H1N1 2009 influenza being due to other viruses or no virus being detected. The use of a highly specific case definition ensured that public health and laboratory resources and public health control activities were targeted at people likely to have the disease and that those unlikely to have the disease were not treated and isolated, or their contacts quarantined unnecessarily. However, the disadvantage of this specific case definition was that a number of people with the disease may have been missed. For example, several samples that tested positive for pandemic H1N1 2009 influenza virus in Greece, where clinicians were allowed more discretion in testing people for influenza, were from people who did not fit the EU case definition [12]. However, because of the statutory system under which all outbreaks of disease, including ILI, are notifiable [3,14] it is unlikely that clusters of indigenous pandemic H1N1 2009 influenza were missed in Ireland.

A challenge with the epidemiological criteria of the case definition was the speed at which countries were becoming affected. In the first few weeks of the pandemic, spread of disease to different countries was rapid and revision of the case definition to include countries where community transmission was occurring proved difficult. This in turn resulted in a lag time between an area being classified as an affected area and people with travel to that area being investigated which may have led to under-identification of cases. A challenge with the clinical criteria of the case definition was that fever was required and subsequent reports from other countries presently indicate that fever is present in a smaller proportion of cases than previously believed and this could further have reduced case identification [11,12].

Our hospitalisation rate of 5% must be interpreted with caution for two reasons. Firstly, in the early phase of the pandemic, in

Figure

Confirmed cases of pandemic H1N1 2009 influenza by source of infection and week of laboratory confirmation, Ireland, 28 April - 18 July 2009 (n=156)

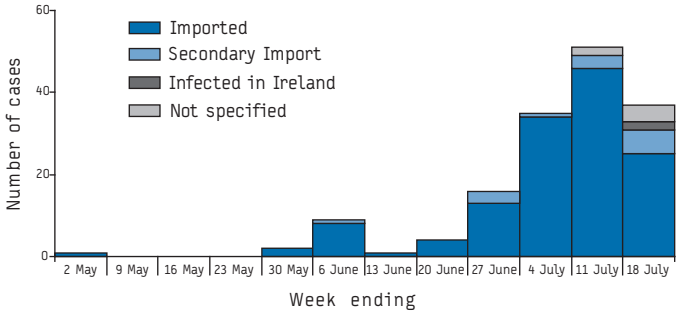

Table 2

Clinical symptoms in confirmed cases of pandemic H1N1 2009 influenza for whom information is available, Ireland, 28 April - 18 July 2009 (n=106)

Symptoms	Number of cases	%
Fever or history of fever	101	95
Sore Throat	64	60
Dry cough	58	55
Myalgia	56	53
Headache	48	45
Rhinorrhoea	36	34
Sneezing	20	19
Diarrhoea	17	16
Arthralgia	16	15
Nausea	15	14
Dyspnoea	14	13
Productive cough	14	13
Vomiting	14	13
Pneumonia	5	5
Altered consciousness	3	3
Conjunctivitis	3	3
Nose bleed	1	1
Seizures	0	0

Ireland, as in other countries [12,15,16], there may initially have been a low threshold for admitting patients with pandemic H1N1 2009 influenza . Reasons for this included concerns as to how the clinical course of patients with a novel disease would progress and for the administration of antivirals to young children, however no patient was admitted purely for infection control. As the pandemic has progressed in other countries there has been a move to hospitalising patients with severe disease only and this has led to much lower hospitalisation rates in those countries [17-19]. Even though there was active follow-up of known cases and their contacts, it is likely that some people with pandemic H1N1 2009 influenza only experienced mild symptoms and thus did not seek medical care which lead to an under-representation of mild cases and hence an over-estimation of hospitalisation rates.

The CIDR surveillance system is the principal infectious disease surveillance system in Ireland and combines clinical and laboratory surveillance data [20]. It was developed to provide high quality timely data and to be flexible to deal with new information and diseases. Once the public health emergency of international concern was declared the system was quickly adapted to include case based and cluster reporting of pandemic H1N1 2009 influenza which was implemented nationally. This was possible because the CIDR system was already functioning well for surveillance of other notifiable diseases. All regions in the country but one had implemented CIDR and surveillance experts in these regions were competent in its use. The CIDR allowed for real-time collection and sharing of data between laboratories, departments of public health and HPSC and enabled real-time analysis of the spread of pandemic H1N1 2009 influenza in the community.

Regional departments of public health undertook contact tracing and collected enhanced surveillance information on all cases under investigation, tasks for which their staff were well experienced as these are often part of processes required to control infectious diseases in the community. This meant that the public health system could respond very quickly to this outbreak. However, the public health workforce is small in Ireland and capacity was stretched to its maximum in responding to the containment phase of the pandemic H1N1 2009 influenza. Ireland moved from containment to mitigation phase on 16 July following advice from the WHO [21]. Once the mitigation phase started, this relieved public health authorities from the burden of intensive contact tracing, and allowed them to focus efforts on case-based surveillance of more severe i.e hospitalised cases and investigation of clusters of disease. At this time there was also a continued focus on increasing public awareness of pandemic H1N1 2009 influenza and encouraging activities to prevent spread of influenza.

While is it impossible to predict how pandemic H1N1 2009 influenza will progress in Ireland, based on other countries' experience and the continuing rise in case numbers in Ireland, it is possible that we will experience a large increase, corresponding to the first wave of a pandemic, in the autumn.

Experience to date internationally has shown that prolonged stays in intensive care units (ICU), for the small proportion of persons needing specialised treatment, have been then main cause of pressure on health services. Currently, enhanced surveillance is being carried out on all hospitalised cases and an ICU enhanced surveillance system is being developed, to monitor those most at risk of developing severe disease. High quality data on hospitalised cases and cases requiring ICU admission is essential to guide health service planning and response to pandemic H1N1 2009 influenza.

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Oseltamivir susceptibility in south-western France during the 2007-8 and 2008-9 influenza epidemics and the ongoing influenza pandemic 2009

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The recent emergence of seasonal influenza A(H1N1) strains resistant to oseltamivir makes it necessary to monitoring carefully the susceptibility of human influenza viruses to neuraminidase inhibitors. We report the prevalence of the oseltamivir resistance among influenza A viruses circulating in south-western France over the past three years: seasonal influenza A(H1N1), seasonal influenza A(H3N2), and the influenza A(H1N1)v viruses associated with the ongoing 2009 pandemic. The main result of the study is the absence of oseltamivir resistance in the pandemic H1N1 influenza strains studied so far (n=129).

Introduction

Even if yearly vaccination remains the best way to prevent influenza, antiviral drugs have proven their efficacy in preventing and treating acute influenza. The adamantanes (amantadine and rimantadine) were the first available influenza antiviral medications. They are associated with severe adverse effects and high levels of resistance among influenza A viruses [1]. This resistance may occur in the absence of antiviral drug use and also emerge rapidly under treatment. Fortunately, neuraminidase inhibitors (NAIs) have been designed to expand the therapeutic possibilities. Presently two anti-influenza drugs are commercially available: oseltamivir and zanamivir [2], which selectively inhibit the neuraminidase of both influenza A and B viruses. Oseltamivir is preferred over zanamivir because it is administered by the oral route [2]. NAIs have been prescribed worldwide since 1999 [3]. In France, their use was limited before the influenza pandemic 2009.

Until recently, the level of resistance to NAIs among circulating influenza A viruses was low [3,4]. However, surveillance studies revealed the sudden emergence of seasonal A(H1N1) strains resistant to oseltamivir in 2007-2008 in Europe where NAIs are used sparsely [5]. From the last quarter of 2007 until June 2008, the highest rate of resistance was reported in Norway (67%). France had the second highest rate with 47% of seasonal A(H1N1) viruses resistant to oseltamivir [6].

Mutations implicated in NAIs resistance were found to be subtype-specific in the neuraminidase active site: The mutations R292K and E119V (in N2 numbering) predominate in the influenza A(H3N2) subtype. R292K induces a resistance to both NAI, whereas E119V leads to oseltamivir but not to zanamivir resistance. H274Y (in N2 numbering) predominates in the seasonal influenza A(H1N1) subtype and confers a high level of resistance to oseltamivir, but these strains remain sensitive to zanamivir [7].

During the season 2007-8, the predominant influenza subtype circulating in south-western France was A(H1N1), while influenza A(H3N2) viruses were the paramount subtype in the 2008-9 winter season. In April 2009, the new influenza A(H1N1)v virus emerged, which has the potential for rapid spread [8]. In the present study, influenza A viruses were collected during two consecutive seasons, 2007-8 and 2008-9, and during the current ongoing influenza pandemic (May to mid-September 2009) for surveillance of oseltamivir resistance using sequence analysis.

Methods

Respiratory samples of patients with influenza-like illness were obtained from Bordeaux Hospital and through a sentinel surveillance network of 21 general practitioners in south-western France. These clinical samples were nasal swabs, bronchoalveolar lavage fluids and nasopharyngeal secretions and were screened by real time RT-PCR in order to determine the virus strain. Primers and probes for the seasonal influenza strains were designed 'in house', those for influenza A(H1N1)v viruses were developed and provided by the two French National Reference Centres for influenza viruses (North and South). None of the patients from whom respiratory specimens were obtained had been treated with NAI before.

The influenza A virus isolates were screened for mutations known to confer resistance to oseltamivir by sequencing of the neuraminidase gene. A multiple sequence alignment was done of influenza A neuraminidase sequences available in Genbank, in order to choose specific RT-PCR primers that would recognise most of the influenza A(H1N1) and A(H3N2) seasonal strains and the pandemic influenza A(H1N1)v. Three primer pairs were designed, targeting the following regions: nucleotide positions 684 to 1,021 of the N1 gene for seasonal influenza A(H1N1) and 692 to 930 for pandemic influenza A(H1N1)v, and nucleotide positions 153 to 1,078 of the N2 gene for seasonal influenza A(H3N2). The target regions were amplified by RT-PCR and sequenced.

The epidemiological features of the ongoing influenza H1N1 pandemic in south-western France were studied following specific instructions from the French Ministry of Health. The target populations were: patients coming from endemic countries (mainly South America and the United States), patients with severe influenza infection, clustered cases of influenza in the community or at school and work place, or pregnant women, children under the age of five months and healthcare workers who had influenza-like symptoms.

Results

In this surveillance study we could amplify sequences for 21 seasonal influenza A(H1N1) viruses in the 2007-8 influenza season, for 97 seasonal influenza A strains (92 H3N2 and five H1N1) in 2008-9, and for 173 pandemic influenza A(H1N1)v viruses collected during the ongoing pandemic. The neuraminidase genes of all 21 seasonal influenza A(H1N1) viruses detected in south-western France during the 2007-8 influenza season were successfully sequenced, and 47.6% of them (10/21) contained a mutation associated with oseltamivir resistance. During the 2008-9 season, none of the 92 seasonal influenza A(H3N2) virus samples contained the E119V or the R292K mutation in the neuraminidase N2 sequence, but all five co-circulating seasonal influenza A(H1N1) viruses had the H274Y mutation in the neuraminidase N1 gene. Since the beginning of the pandemic in late April 2009, 173 confirmed cases of pandemic influenza A(H1N1)v have been found in south-western France. Only 129 of those isolates have been genotyped so far. According to their neuraminidase sequence, all 129 were found to be sensitive to oseltamivir (Table 1). Currently, influenza A(H1N1) 2009 incidence is increasing worldwide including in south-western France (Table 2). As already described, young adults (19-34 years) seem to be particularly sensitive to A(H1N1) 2009 infection (Figure).

Table 1

Oseltamivir resistance in influenza A isolates collected since 2007 in south-western France (n=247)

	Number of samples genotyped	Number of oseltamivir-resistant samples
2007-2008	21 A(H1N1) seasonal	10
2008-2009	5 A(H1N1) seasonal	5
	92 A(H3N2) seasonal	0
1 May – 15 September 2009	129 A(H1N1) 2009 pandemic	0

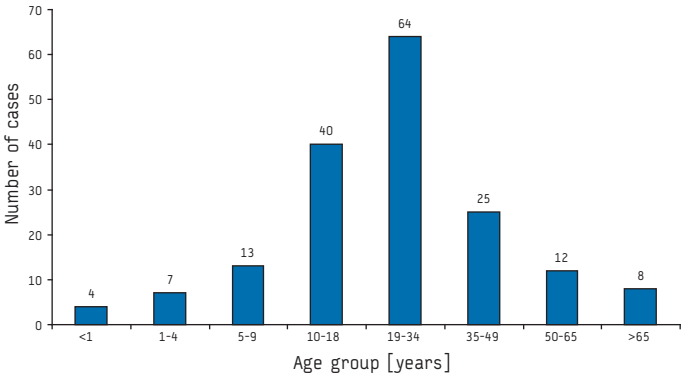
Table 2

Prevalence of pandemic influenza A(H1N1)v prevalence in south-western France, 1 May to 15 September 2009 (n=173)

	May	June	July	August	Sept,	Total
Number of samples tested	31	36	93	410	302	**872**
Number of influenza A(H1N1)v cases	3	9	8	113	40	**173**
Positive ratio (%)	9,7	25,0	8,6	27,6	13,2	**19,8**

Figure

Age distribution of cases of pandemic influenza A(H1N1)v, south-western France, 1 May – 15 September 2009 (n=173)



Discussion

As we had no phenotypic data in this study, we could not observe potential new mutations leading to resistance. Therefore, this study is limited to previously described resistance mutations that can be shown by sequencing. We report the results of a surveillance study for NAIs susceptibility among influenza A viruses isolated in south-western France during the last two influenza seasons and the current 2009 pandemic. Results obtained in the 2007-8 and 2008-9 influenza seasons are in accordance with the World Health Organization's Global Influenza Surveillance Network data. The recent emergence of oseltamivir-resistant influenza A(H1N1) strains during 2007-8 season in western Europe may appear surprising in view of the small proportion of treated patients [9]. This could have dramatic consequences if resistance were to emerge also among avian influenza A(H5N1) viruses or pandemic influenza A(H1N1)v strains. To date, only 12 oseltamivir-resistant influenza A(H1N1)v viruses have been detected worldwide, namely in Canada, China, Denmark, Hong Kong, Japan, Singapore and the United States [10]. Oseltamivir has been recommended since the beginning of the influenza pandemic 2009 for treatment and prophylaxis. Monitoring the susceptibility of pandemic influenza viruses to oseltamivir is important to identify cases in which zanamivir should be used as an alternative drug.

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Trichinellosis outbreak in Lithuania, Ukmerge region, June 2009

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An outbreak of trichinellosis due to wild boar meat was detected in Lithuania in June 2009. The outbreak affected 107 people all of whom had consumed sausages made of wild boar meat. Inspection of food samples confirmed the presence of *Trichinella* larvae in the meat.

Background

Several human cases of trichinellosis are reported in Lithuania every year. Between 1999 and 2008, a total of 359 cases were registered, including 66 sporadic cases and 42 outbreaks. During these ten years the incidence of trichinellosis decreased from 1.7 to 1.2 cases per 100,000 population [1].

The epidemiological investigations show that human trichinellosis in Lithuania is mostly spread by consumption of meat from infected pigs and wild boars. Of all outbreaks reported from 1999 to 2008, 58% occurred due to consumption of meat from home-raised pigs, 10% due to infected wild boar meat and about 8% due to illegal sale of meat. Some 24% of outbreaks were unexplained.

Outbreak investigation

On 11 June 2009 the Lithuanian Centre for Communicable Disease Prevention and Control received an urgent report about five suspected cases of human trichinellosis in Ukmerge municipality. An epidemiological investigation was started on the same day in order to determine the extent of the outbreak, identify its source and propose control measures. The investigations involved specialists from the Ukmerge department at Vilnius public health centre and the Ukmerge district State Food and Veterinary service.

Case finding

A standardised questionnaire was used to collect information on the clinical features, date of onset of symptoms, consumption of meat products, and dates and places of meat purchase. Investigation of the first cases quickly revealed that they had consumed homemade sausages from wild boar.

A **confirmed case** was defined as a person with the following clinical symptoms: fever (> 38 °C), with myalgia, or facial or orbital oedema, who had consumed homemade sausages from wild boar, produced on 16 May 2009, and had positive serology for *Trichinella*. A **probable case** was defined as a person with the following clinical symptoms: fever, myalgia, facial or orbital oedema, or hypereosinophilia, who had consumed homemade sausages from wild boar, produced on 16 May 2009. A **suspected case** was defined as a person with hypereosinophilia alone or associated with fever, myalgia or orbital oedema, who had consumed homemade sausages from wild boar, produced on 16 May 2009.

Patients and their family members were interviewed and active finding of persons who had consumed suspected meat was implemented after receiving an urgent report from a personal healthcare institution (general practice and hospital) about a suspected case of trichinellosis. Active case finding was started every time a healthcare institution reported a suspected trichinella case. Persons who had consumed suspected meat were referred to their local healthcare institutions for laboratory examination and medical observation. Blood samples were tested for eosinophilia and serological investigations for antibodies against trichinellosis were performed by ELISA.

Food investigation

On 11 June the State Food and Veterinary service collected the remainder of wild boar sausages (13.4 kg produced from several animals) from hunters and their family members and tested them for trichinellosis. The food samples were tested using the artificial digestion method.

Results

Human cases

As a result of the investigations, it was established that 128 persons had consumed sausage made from wild boar meat suspected as the source of infection. Of these, 107 people were considered to have been affected by the outbreak. Fourteen cases (13.1%) were laboratory-confirmed, the remaining 93 (86.9%) were regarded as probable cases fulfilling clinical and epidemiological criteria. Blood serological reactions for the detection of antibodies against *Trichinella* were performed three to four weeks after meat consumption. It is presumed that this time was too short for finding antibodies against *Trichinella*, which can explain the relatively small proportion of confirmed cases.

The first patient fell ill on 20 May; the last case was detected on 26 June (Figure 1). The outbreak lasted 37 days. The shortest incubation period was five days and the longest was 25 days.

Most cases were reported from Ukmerge municipality but the infection spread beyond this region, affecting six municipalities in total (Table).

The majority of cases were adults (88.8 %), only 12 cases in children were registered (Figure 2).

The main clinical symptoms were: fatigue (100%), nausea (94.6%), fever (91.6%), muscular pain (88.2%), facial oedema (52.3%), orbital oedema (946%), and haemorrhagic rash of the skin (14.6%). Eosinophilia was found in all patients. The clinical symptoms of disease were serious in five cases (4.7%), medium in 50 cases (46.7%) and mild in 52 cases (48.6%). A severe course of disease was defined by fever higher than 39 °C, face swelling, pain of neck, shoulders and trunk, myalgia, and neurological complications (lethargy, apathy and excitement). A medium course of disease was defined by fever of up to 39 °C, orbital oedema and lesser myalgia. A mild course of disease was defined by subfebril temperature and insignificant orbital oedema.

Fifty-five patients (51.4%) were hospitalised. The patients were treated by mebendazole. Corticosteroides were administered for patients with a severe and medium course of disease. All patients were followed up after treatment and all recovered. Persons with a severe or medium course of disease will be followed for six months by their local healthcare institutions. For these patients, an assessment of eosinophilia and myalgia will be done regularly.

Food source

On 12 June the laboratory department of the State Food and Veterinary Risk Assessment Institute found *Trichinella* pathogens in the collected sausage samples. In 1g of meat about 20 larvae were found. The samples will be sent to Italy for the determination of the *Trichinella* type.

Conclusion

The source of infection was identified to be wild boar meat. Several wild boars were hunted on 10 May 2009 in Ukmerge region. The meat was not inspected for the presence of *Trichinella*. On 16 May 2009, 50 kg of cold-smoked sausages were produced from the wild boar meat in a joint stock company 'Alekniskis'. The sausages were not produced for trade but only for private consumption. The sausages were distributed to huntsmen who ate this meat themselves and distributed it further among their family members, neighbours, relatives and acquaintances.

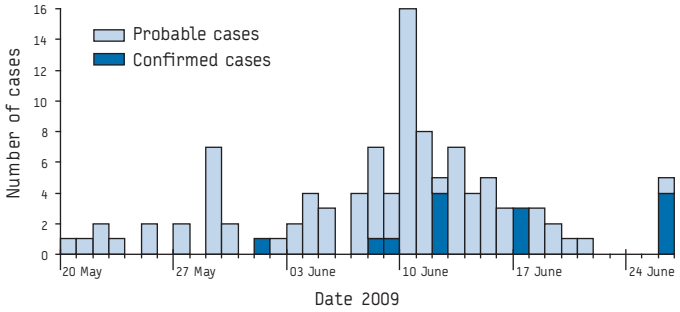
It is believed that a large number of cases were due to the significant invasion of *Trichinella* larvae in the meat.

Wild boar meat is the second most common cause of trichinellosis in Lithuania. Another larger outbreak had been registered in 2001, in which 65 persons fell ill with trichinellosis (69 cases or 65% of all cases in 2001). Investigations performed in 2000-2002 showed that about 0.5% of wild boars in Lithuania are infected with *Trichinella* [2]. The infected animals are evenly distributed in the whole country. Therefore the meat of wild boar remains an important source of infection in Lithuania.

According to legislation in Lithuania all slaughtered pigs and hunted wild boars must be examined for *Trichinella*. Presently there are two methods used to detect *Trichinella* in meat: trichinoscopy (compressorium) and artificial digestion method. Epidemiological data suggests that in spite of these regulations, consumption of uninspected meat still occurs. Therefore, intensive public education, especially for small pig breeders and hunters, is needed in order to prevent human trichinellosis in Lithuania.

FIGURE 1

Cases of trichinellosis reported in an outbreak in Lithuania, May-June 2009, by date of disease onset (n=107)



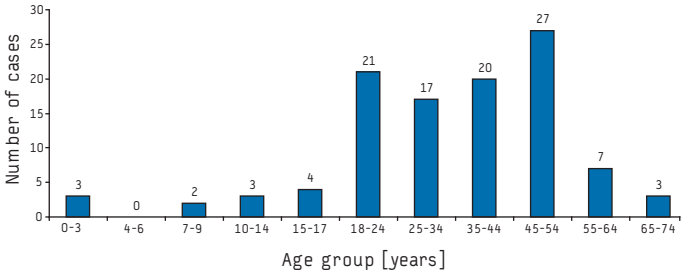
TABLE

Geographical distribution of cases of trichinellosis, Lithuania, May-June 2009, by municipality (n=107)

Municipality	Number of cases (%)
Ukmerge	56 (52.3%)
Vilnius	9 (8.4%)
Kaunas	16 (15.0%)
Kedainiai	21 (19.6%)
Jonava	4 (3.7%)
Zarasai	1 (0.9%)
Total	**107 (100%)**

FIGURE 2

Age distribution of cases of trichinellosis, Lithuania, May-June 2009 (n=107)



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Molecular characterisation of PFGE non-typable methicillin-resistant Staphylococcus aureus in the Netherlands, 2007

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In 2007 in the Netherlands, 30% of all human isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) sent to the National Institute for Public Health and the Environment could not be typed by pulsed-field gel electrophoresis (non-typable (NT)-MRSA). Molecular characterisation of the NT-MRSA isolates revealed 27 different spa types and two distinct SCC*mec* types, type IV and V. All NT-MRSA isolates were closely related based on spa and multi-locus sequence typing and belonged to the ST398 lineage. The rapid increase of NT-MRSA (ST398) isolates over the last years shows the importance of this relatively new clonal lineage.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen causing not only infections in the hospital but also in the community. Lately, the epidemiology of MRSA is changing, focusing more and more on community-associated (CA-) MRSA. CA-MRSA are often Panton-Valentine leukocidin (PVL)-positive and can cause serious skin and soft-tissue infections, but also conditions such as necrotising pneumonia [1].

Since 2005, pigs have been identified as a possible new reservoir for MRSA [2,3]. Although the pig-related MRSA isolates are almost always PVL-negative, they could be considered as CA-MRSA. Pig-related MRSA strains can easily be identified by *Sma*I pulsed-field gel electrophoresis (PFGE). These strains possess a methylation enzyme that methylates the *Sma*I restriction sites. Consequently, no banding pattern is obtained in the PFGE and no PFGE type can be assigned [4]. Furthermore, staphylococcal protein A (*spa*) typing has shown that the pig-related strains belong to specific spa types, indicating a clonal structure. This clonality was also supported by multi-locus sequence typing (MLST), since the pig-related strains were all sequence type (ST) 398 or single locus variants of ST398 (ST752 and ST753) [5]. To date, the pig-related MRSA has also been found in other farm animals such as

Table 1

Incidence of *spa* types of PFGE NT-MRSA isolates in the Netherlands in 2007 (n=793)

Spa type	No. of isolates*	% of NT-MRSA*	% of all isolates*
t011	370	46.7	14.1
t108	268	33.8	10.2
t567	42	5.3	1.6
t034	20	2.5	0.8
t899	19	2.4	0.7
t571	15	1.9	0.6
t2330	11	1.4	0.4
t2123	8	1.0	0.3
t1456, 2383	5 each	0.6	0.2
t1255, t3013	4 each	0.5	0.2
t588, t1184, t1457	3 each	0.4	0.1
t2582	2	0.3	0.1
t779, t943, t1451, t2287, t2329, t2748, t2971, t3014, t3053, t3146, t3208	1 each	0.1	0.0
Total	**793**	**100**	**30.3**

* Data per *spa* type
NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*; PFGE: pulsed-field gel electrophoresis.

horses [6], poultry [7], and is associated with cattle [5]. Therefore, it is no longer considered a pig-related MRSA clone but a livestock-related clonal lineage.

In January 2003, the first MRSA was found in the Netherlands which could not be typed by PFGE. From then on it was referred to as PFGE non-typable (NT)-MRSA. In the following years, the number of NT-MRSA increased rapidly and due to the relation of NT-MRSA with pigs, several studies were performed in order to get an idea about the MRSA carriage rate of pigs and pig farmers [2,5,8-10]. Upon the first results it became clear that pigs could be considered as a reservoir for MRSA and pig-to-human transmission had occurred [11]. Although rarely seen to date, human infections due to ST398 MRSA have occurred [12-14]. Based on the results of the NT-MRSA studies, the Dutch national MRSA guidelines were adjusted in July 2006 and November 2007 to the effect that all individuals working or living in close contact with living pigs or cattle are isolated and screened for MRSA upon admission to a hospital. As a result of this new guideline the number of detected NT-MRSA isolates increased.

In the Netherlands, all MRSA isolates are sent to the national MRSA reference centre. The present paper gives an overview of NT-MRSA in the Netherlands in 2007, the molecular characteristics of NT-MRSA and the clonal structure of these isolates. The data will show the importance of this relatively new CA-MRSA clonal lineage.

Methods

Bacterial isolates

In the Netherlands, the National Institute for Public Health and the Environment (RIVM) serves as the national reference centre for surveillance of MRSA. All first MRSA isolates of newly identified carriers, one per patient, are sent to the RIVM for typing. All MRSA isolates submitted in 2007 were used for typing.

Typing

All MRSA isolates were typed by PFGE using *Sma*I as the restriction enzyme according to the HARMONY PFGE protocol [15]. The isolates that could not be typed by PFGE were selected and used for *spa* typing [16]. New spa types were assigned with Ridom Staphtype software version 1.5.13 (Ridom GmbH). For the analysis of *spa* types and the creation of a minimum spanning tree, Bionumerics software version 5.1 (Applied Maths) was used. The PVL genes were detected by PCR according to the method described by Lina *et al.* [1]. The first 300 NT-MRSA isolates of 2007 were used to determine the SCCmec type by multiplex PCR according to Kondo et al. [17]. In case the *spa* type of an isolate was different from those found among the first 300 NT-MRSA isolates, it was also subjected to SCC*mec* typing. Thus, a total of 308 isolates were analysed for SCC*mec* type. Furthermore, at least one isolate per spa type was subjected to MLST [18].

Results

In 2007, the RIVM received 2,619 unique MRSA isolates, of which 793 (30.3%) were non-typable by PFGE. *Spa* typing of these NT-MRSA isolates revealed 27 different *spa* types. Table 1 shows the incidence of the different *spa* types.

Two dominant *spa* types, t011 and t108, accounted for 80% of all NT-MRSA isolates and eleven *spa* types were only found once. In order to determine whether the 27 different *spa* types were related to each other, the repeats of each *spa* type were aligned (Table 2), and a minimum spanning tree was made based on the *spa* types of the isolates (Figure).

All *spa* types were closely related, as confirmed by the MLST results. All isolates belonged to ST398. Two different SCC*mec* types were found among the 308 NT-MRSA that were tested: SCC*mec* type IV (n= 78) and type V (n=198) (Table 3).

For 32 isolates the SCC*mec* type could not be determined. For *spa* type t011, 139 NT-MRSA isolates were tested and were of SCC*mec* type IV (n=62) and type V (n=64), or could not be typed (n=13). Surprisingly, 98 isolates with *spa* type t108 were either SCC*mec* type V (n=93) or could not be typed (n=5). No SCC*mec* type IV was found among these 98.

Only one NT-MRSA isolate was PVL positive. This isolate had *spa* type t034 and the SCC*mec* PCR resulted in SCC*mec* type V. The PVL PCR was retested on this isolate and PVL was confirmed.

Table 2

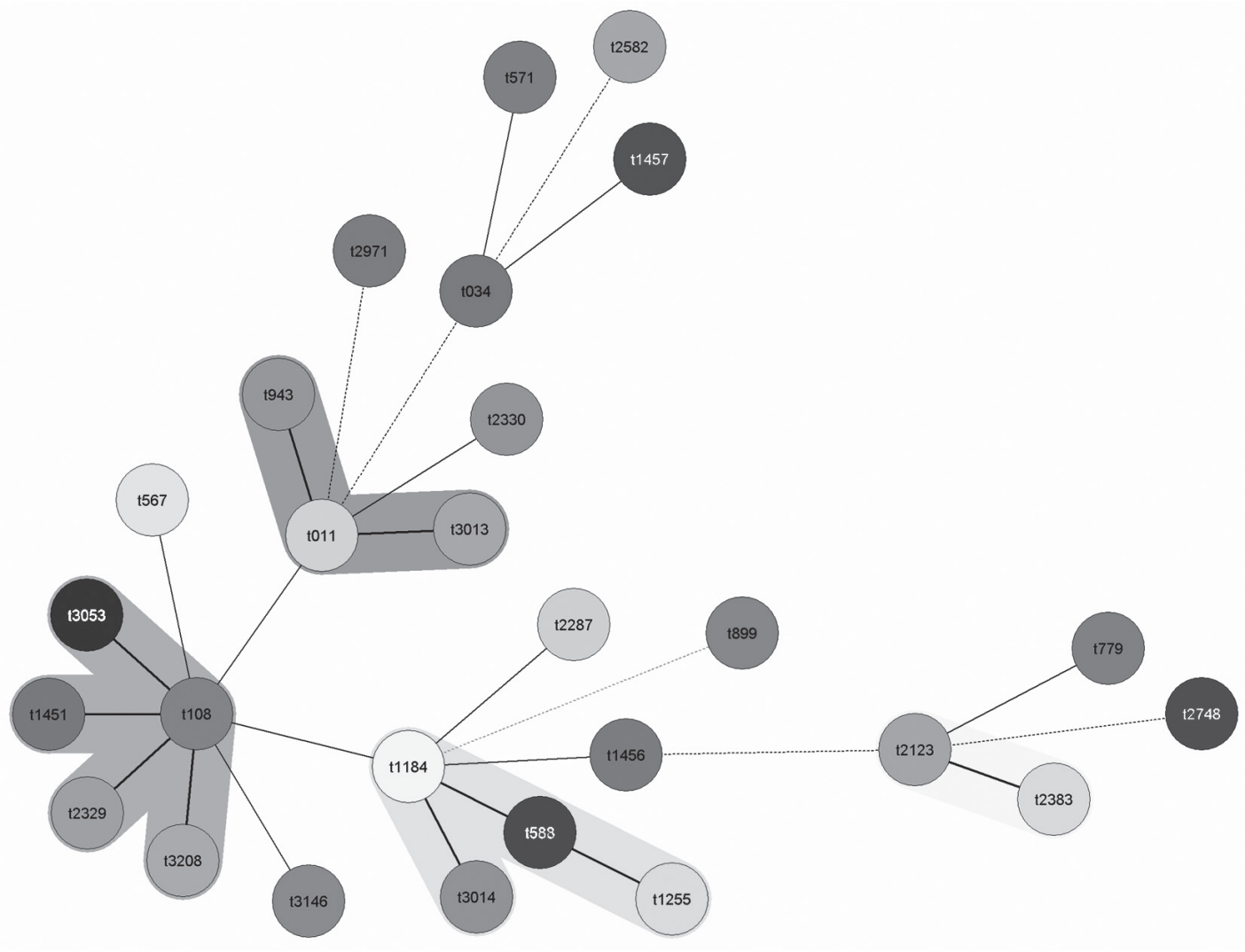
Alignment of tandem repeats* of NT-MRSA *spa* types, Netherlands, 2007

spa	repeats													
t011	8	16	-	2	25	-	-	-	-	-	34	24	25	-
t034	8	16	-	2	25	-	2	25	-	-	34	24	25	-
t108	8	16	-	2	25	-	-	-	-	-	-	24	25	-
t567	8	-	-	2	25	-	-	-	-	-	-	24	25	-
t571	8	16	-	2	25	-	2	25	-	-	34	-	25	-
t588	8	16	-	2	-	-	-	-	-	-	-	24	25	-
t779	8	-	-	-	-	-	-	-	-	-	-	-	-	-
t899	7	16	23	2	-	-	-	-	-	-	34	-	-	-
t943	8	16	-	2	25	-	-	25	-	-	-	24	25	-
t1184	8	16	-	2	25	-	-	-	-	-	-	-	25	-
t1255	8	16	-	-	-	-	-	-	-	-	34	24	25	-
t1451	8	16	-	2	25	-	-	-	-	-	34	-	25	-
t1456	8	16	-	2	25	-	-	-	-	-	-	-	-	-
t1457	8	16	-	2	25	34	2	25	-	-	34	24	25	-
t2123	8	-	-	-	25	-	-	-	-	-	-	-	-	-
t2287	8	-	-	2	25	-	-	25	-	-	-	-	-	-
t2329	8	16	-	159	25	-	-	-	-	-	-	24	25	-
t2330	8	16	-	2	25	-	-	-	-	-	34	24	25	25
t2383	8	16	-	-	-	-	-	-	-	-	-	-	-	-
t2582	8	16	-	2	25	-	2	25	2	25	34	24	25	-
t2748	26	-	-	-	-	-	-	-	-	-	-	24	25	-
t2971	8	-	23	-	25	-	-	-	-	-	34	24	25	-
t3013	8	16	-	-	-	34	-	25	-	-	34	24	25	-
t3014	8	16	-	2	65	-	-	25	-	-	-	-	-	-
t3053	8	16	-	2	65	-	-	-	-	-	-	24	25	-
t3146	8	16	-	2	-	-	-	-	-	-	-	24	25	25
t3208	8	16	-	2	25	-	-	-	-	-	-	24	24	-

* Repeats with only one base difference are mentioned in the same column, e.g. repeat 7, 8, and 26. Repeat 16 and 23 also differ in one base but because they were are also found together in *spa* type t899 they were put into a separate column.
NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*.

Figure

Genetic relatedness of 793 NT-MRSA isolates, represented as a minimum spanning tree based on *spa* types



The *spa* types are displayed as circles. Clustering was performed using the Bionumerics *spa*-plugin with a conservative alignment setting of 100% and 0% maximum duplication length. The halos surrounding the circles indicate related *spa* types.
NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*.

Table 3

SCCmec type of PFGE NT-MRSA isolates in the Netherlands in 2007 (n=308)

	spa type (n)
SCC*mec* type IV (n=78)	t011 (62), t034 (1), t571 (1), t899 (5), t1451 (1), t2123 (4), t2383 (1), t2748 (1), t2971 (1), t3013 (1)
SCC*mec* type V (n=198)	t011 (64), t034 (5), t108 (93), t567 (5), t571 (5), t588 (2), t779 (1), t899 (8), t943 (1), t1184 (1), t1255 (1), t1456 (1), t1457 (2), t2123 (1), t2329 (1), t2330 (3), t3014 (1), t3053 (1), t3146 (1), t3208 (1)
unknown SCC*mec* type (n=32)	t011 (13), t034 (4), t108 (5), t567 (8), t2287 (1), t2582 (1)
total (n=308)	

NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*; PFGE: pulsed-field gel electrophoresis.

Discussion

In the year 2003 the first MRSA was found in the Dutch national MRSA surveillance which could not be typed by PFGE. At that time the correlation between animals and NT-MRSA was unknown. All MRSA isolates in 2002 had been typable by PFGE. The importance of NT-MRSA became clear when an increasing number of these isolates were observed. After the MRSA screening guidelines were adjusted to include screening of all hospitalised patients who had close contact with living pigs or cattle, even more NT-MRSA isolates were recorded. In 2007, the NT-MRSA clone accounted for 30% of all MRSA isolates sent to the Dutch national reference centre (see Table 1). The MRSA isolates included in this study were those found through screening of patients in healthcare settings. NT-MRSA isolates found in research programmes were excluded.

Molecular characterisation of all NT-MRSA isolates from 2007 (n=793) showed 27 different *spa* types and two different SCC*mec* types. Two dominant *spa* types, t011 and t108, accounted for 80% of all NT-MRSA isolates. Eleven *spa* types were found only once. All *spa* types formed one clonal *spa* cluster (see Table 2 and Figure). MLST was performed on at least one isolate of each *spa* type. All isolates were of the ST398 lineage, indicating the clonal structure of these isolates.

The PVL prevalence of all MRSA isolates found in 2007 was 12% (data not shown). However, only one of the NT-MRSA isolates gave a product in a PCR for the PVL genes. Infection with the bacteriophage carrying the genes for PVL seems to be less common in the ST398 lineage. Nevertheless, several reports about infections [12-14] and one outbreak [19] caused by the ST398 lineage have recently been published. Furthermore, methicillin-susceptible ST398 *S. aureus* (MSSA) have been isolated from three human cases with bacteraemia [20], indicating that the ST398 MRSA lineage could pose a serious threat to public health if it retained the virulence of ST398 MSSA.

SCC*mec* typing of the NT-MRSA isolates revealed two SCC*mec* types, IV and V. These two SCCmec types are considered to be community-associated. Furthermore, 32 isolates could not be typed by SCC*mec* , indicating the emergence of one or more new SCC*mec* type(s). Remarkable is the difference between the two most prevalent spa types, t011 and t108. In MRSA isolates with spa type t011, both SCC*mec* types were found, whereas in isolates with *spa* type t108, only SCC*mec* type V was detected. It is conceivable that MRSA *spa* type t108 SCC*mec* type V may have originated from MRSA *spa* type t011 SCC*mec* type V by deletion of repeat 34. Since SCC*mec* is a mobile genetic element that carries the *mec*A gene, it is also possible that MRSA isolates could acquire different SCC*mec* types.

The increasing number of articles concerning the ST398 clone is an indication of the international emergence of this clone. In some European countries, as in the Netherlands, *spa* type t011 also dominates [21,22], while other countries reported other *spa* types as the dominant type [13,23,24]. Outside Europe, ST398 MRSA has also been found in the United States [25,26], Canada [9], Singapore [27], and China [28]. Generally, ST398 isolates of human and animal origin showed similar molecular characteristics, being non-typable by *Sma*I PFGE and having closely related *spa* types, and SCC*mec* type IV, V, or variants thereof.

Today, the ST398 MRSA lineage is no longer specifically seen in pigs, but has also been found in other animals such as horses [6], poultry [7], dogs [13], and associated with cattle [5]. A Dutch study on the prevalence of MRSA in pigs identified *spa* types similar to the human *spa* types found in this study [2]. Currently, people in close contact with living pigs and cattle are screened upon admission to the hospital. Perhaps these guidelines will need to be adjusted again in the near future, since more animal species seem to serve as a reservoir for MRSA. Whether the number of NT-MRSA will increase even further remains to be seen. In the Netherlands, a country with a low prevalence of MRSA, transmission of MRSA within a clinical setting could be difficult to predict, as more than 80% of the NT-MRSA have either *spa* type t011 or t108.

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