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Dengue and other *Aedes*-borne viruses: a threat to Europe?

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At the beginning of the 20th century, dengue outbreaks were rather common in the Mediterranean basin. The last major epidemic on the European continent occurred in 1927/28 and predominantly affected Athens and neighbouring areas of Greece. After a first mild wave, which nearly ended with the arrival of cold weather in the winter season, a small number of cases continued to occur through the winter and spring, increasing dramatically in August 1928 [1-3]. It is conceivable that both the virus and its primary vector, the *Aedes aegypti* mosquito, survived the winter in the city, inside heated houses. Serological surveys detected neutralising antibodies to different dengue virus (DENV) serotypes in samples of individuals living in Athens in that period [4,5]. Some time after this severe outbreak, with 1,000 to 1,500 deaths, both dengue and its primary vector 'abandoned' the European continent.

The outbreak of seven autochthonous dengue cases reported by Succo et al. in this issue of *Eurosurveillance* [6] was triggered by one infected traveller returning from French Polynesia in the summer of 2015, and occurred in an area where another vector, *Ae. albopictus*, the Asian Tiger mosquito, was established in 2005.

This is not the first event of local transmission of DENV reported in Europe in recent years. Since 2010, at least 23 dengue cases were detected. In September 2010, two autochthonous cases of dengue fever were identified in Nice, southern France. The index case had friends from the West French Indies staying with him, while the second case was an individual living nearby [7]. In the summer of the same year, another transmission event occurred in Croatia [8,9]. The index case was a German man returning in mid-August from a two-week holiday spent at the Peljesac peninsula and the isle of Korĉula, ca 100 km north-west of Dubrovnik. A second autochthonous case, and other 15 individuals with serological evidence of recent infection, were identified in October 2010. How the virus was introduced in Croatia remains unclear. In 2013 and 2014, five autochthonous case of dengue were identified in southern France, one in Bouches-du-Rhône (2013) [10], and four in Aubage and Toulon-Hìres (2014) [11]. *Ae. albopictus* was the vector in all the transmission events listed here.

Dengue is not the only *Aedes*-borne viral disease threatening the health of European citizens. Nearly 10 years ago, in the summer of 2007, more than 250 cases of chikungunya occurred in the north-east of Italy [12]. The primary case was a viraemic individual arriving from the Indian State of Kerala. The chikungunya virus (CHIKV) implicated in the sustained outbreak carried the A226V mutation, which increases virus fitness and is usually detected in areas where the Tiger mosquito is the predominant vector [13]. In September 2010, autochthonous transmission of the CHIKV was also identified in south-east France, where chikungunya was diagnosed in two children living in the same area as another child who developed a febrile illness after returning from Rajasthan, India [14].

At present, there is concern about the possible emergence of Zika virus, which has been recently declared a 'Public Health Emergency of International Concern' by the World Health Organization [15]. Whether the increased risk of mosquito-borne transmission during the summer season in Europe will materialise in form of *Aedes*-borne autochthonous cases of Zika virus infections is unknown.

With the exception of a large dengue outbreak with over 2,100 cases that occurred from October 2012 to March 2013 in the subtropical archipelago of Madeira, located in the Atlantic Ocean at around 1,000 km from mainland Portugal, where *Ae. aegypti* is largely predominant [16], the vector involved in local transmission of DENV and CHIKV in Europe has always been *Ae. albopictus*.

The importance of *Ae. albopictus* is constantly growing as a consequence of rapid changes in its overall distribution and virus adaptation to the vector [17]. Since the time of World War II, the Tiger mosquito was involved in several dengue and chikungunya outbreaks that occurred in Japan, Hawaii, southern China, Indian Ocean Islands, and the Indian sub-continent [18].

In temperate areas, the global spread of *Ae. albopictus* is a prerequisite for transmission. Furthermore, several factors may increase the risk of importation of dengue and similar mosquito-borne infections into previously disease-free areas, well beyond the tropical and subtropical belt, where the vector is present: (i) the massive increase of mosquito-borne infections such as dengue, in certain areas of the world, driven by rapid population growth and uncontrolled urbanisation [19]; (ii) the spread of dengue, chikungunya, and Zika viruses in many touristic destinations in southeast Asia, Indian Ocean Islands, Pacific Islands, and in particular Central and South America; (iii) increased human mobility, which is an important driver of long-distance virus transportation.

The article by Succo et al. is an additional example that dengue transmission can occur in Europe However, to what extent tropical vector-borne infections may cause large outbreaks or even become endemic in Europe cannot be easily predicted. In a likely scenario, autochthonous cases may appear once the virus is introduced and amplified by local mosquitoes in a permissive environment. However, implementation of vector control measures following early detection of cases, combined with the decline of mosquito activity at the beginning of the winter season, may cut down the basic reproductive number (R^0) and to stop transmission.

To better assess the risk of sustained transmission and persistence of *Aedes*-borne infections in Europe, the characteristics of the vector and the influence of climatic factors should be considered. *Ae. albopictus* adapts better than *Ae. aegypti* to temperate climate and may be implicated in outbreaks in areas where *Ae. aegypti* is not established. However, *Ae. albopictus* usually feeds on a single individual while *Ae. aegypti* tends to feed on more individuals during one gonotrophic cycle and only on humans. Thus, outbreaks caused by *Ae. albopictus*, may be more limited in size that those cause by Ae. aegypti, even if vector density is similar [17,18]. Moreover, vertical transmission of DENV and CHIKV from mosquitoes to their offspring is not very efficient. The low efficiency of transovarial transmission combined with the decline of mosquito activity during the cold season may explain the self-limiting nature of outbreaks occurring in temperate climate areas. Finally, even though DENV and Zika fitness for *Ae. albopictus* is not negligible, it is lower than for *Ae. aegypti* [20,21]; thus the sustainability of DENV, ZIKV and, to a lesser extent, CHIKV variant transmission, in areas where *Ae. albopictus* is the predominant vector, is not likely to be high.

Some of the consideration reported above may appear reassuring. However, the likelihood of future occurrence of dengue and other *Aedes*-borne viruses in Europe will be impacted by (i) repeated introduction of the infection, (ii) climate change, which may favour overwintering of virus and mosquitoes, (iii) possible increased fitness of viruses for the Tiger mosquito, as happened for CHIKV, and (iv) the return of *Ae. aegypti*, which is now established Caucasian cost of the Black Sea, where it competes with *Ae. albopictus* and *Ae. koreicus* [22]. To this regard, further expansion of *Ae. aegypti* towards the Mediterranean shores may not be fully excluded.

The article by Succo et al., published in this issue of *Eurosurveillance*, confirms the potential risk represented by dengue and other *Aedes*-borne scourges to Mediterranean Europe, underlining the importance of risk assessment, enhanced surveillance aimed at early detection of transmission chains, and mosquito control programs. Though the risk of large scale outbreaks and endemicity may appear rather low for most European countries, the effect of environmental, ecological, entomological, demographic, and behavioural changes on the epidemic potential of exotic *Aedes*-borne infections should not be underestimated.

Conflict of interest

None declared.

References

1. Cardamatis JP. La dengue in Greece.Bull Soc Pathol Exot. 1929;22:272-92.
2. Papaevangelou G, Halstead SB. Infections with two dengue viruses in Greece in the 20th century. Did dengue hemorrhagic fever occur in the 1928 epidemic?J Trop Med Hyg. 1977;80(3):46-51.PMID: 327086
3. Schaffner F, Mathis A. Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. Lancet Infect Dis. 2014;14(12):1271-80.DOI: 10.1016/S1473-3099(14)70834-5 PMID: 25172160
4. Theiler M, Casals J, Moutousses C. Etiology of t e 1927-28 epidemic of dengue in Greece.Proc Soc Exp Biol Med. 1960;103(1):244-6.DOI: 10.3181/00379727-103-25474 PMID: 13837683
5. Halstead SB, Papaevangelou G. Transmission of dengue 1 and 2 viruses in Greece in 1928.Am J Trop Med Hyg. 1980;29:635-7.
6. Succo T, Leparc-Goffart I, Ferré J, Roiz DBroche BMaquart M et al. Autochthonous dengue outbreak in Nîmes, South of France, July to September 2015.Euro Surveill. 2016;21(21):30240. DOI: 10.2807/1560-7917.ES.2016.21.21.30240
7. La Ruche G, Souarès Y, Armengaud A, Peloux-Petiot F, Delaunay P, Desprès P, et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveill. 2010;15(39):19676.PMID: 20929659
8. Schmidt-Chanasit J, Haditsch M, Schöneberg I, Günther S, Stark K, Frank C. Dengue virus infection in a traveller returning from Croatia to Germany.Euro Surveill. 2010;15(40):19677. PMID: 20946759
9. Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobučar A, Pem-Novosel I, et al. Autochthonous dengue fever in Croatia, August-September 2010. Euro Surveill. 2011;16(9):19805.PMID: 21392489
10. Marchand E, Prat C, Jeannin C, Lafont E, Bergmann T, Flusin O, et al. Autochthonous case of dengue in France, October 2013. Euro Surveill. 2013;18(50):20661.DOI: 10.2807/1560-7917.ES2013.18.50.20661 PMID: 24342514
11. Giron S, Rizzi J, Leparc-Goffart I, Septfons A, Tine R, Cadiou B , et al. New occurrence of autochthonous cases of dengue fever

in Southern France, August-September 2014. Bull Epidemiol Hebd (Paris). 2015;13-14:217-23.

12. Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, et al. , CHIKV study group. Infection with chikungunya virus in Italy: an outbreak in a temperate region.Lancet. 2007;370(9602):1840-6.DOI: 10.1016/S0140-6736(07)61779-6 PMID: 18061059
13. Vazeille M, Moutailler S, Coudrier D, Rousseaux C, Khun H, Huerre M, et al. Two Chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, Aedes albopictus. PLoS One. 2007;2(11):e1168.DOI: 10.1371/journal.pone.0001168 PMID: 18000540
14. Grandadam M, Caro V, Plumet S, Thiberge JM, Souarès Y, Failloux AB, et al. Chikungunya virus, southeastern France. Emerg Infect Dis. 2011;17(5):910-3.DOI: 10.3201/eid1705.101873 PMID: 21529410
15. World Health Organization (WHO). WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations. Geneva: WHO; 1 February 2016. Available from: http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/
16. Alves MJ, Fernandes PL, Amaro F, Osório H, Luz T, Parreira P, et al. Clinical presentation and laboratory findings for the first autochthonous cases of dengue fever in Madeira island, Portugal, October 2012. Euro Surveill. 2013;18(6):20398.PMID: 23410256
17. Rezza G. Aedes albopictus and the reemergence of dengue. BMC2012; 12:72.
18. Rezza G. Dengue and chikungunya: long-distance spread and outbreaks in naïve areas.Pathog Glob Health. 2014;108(8):349-55.DOI: 10.1179/2047773214Y.0000000163 PMID: 25491436
19. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21(st) Century.Trop Med Health. 2011;39(4) Suppl;3-11.DOI: 10.2149/tmh.2011-S05 PMID: 22500131
20. Moutailler S, Barré H, Vazeille M, Failloux AB. Recently introduced Aedes albopictus in Corsica is competent to Chikungunya virus and in a lesser extent to dengue virus.Trop Med Int Health. 2009;14(9):1105-9.DOI: 10.2807/1560-7917.ES.2016.21.15.30199 PMID: 19725926
21. Di Luca M, Severini F, Toma L, Boccolini D, Romi R, Remoli ME, et al. Experimental studies of susceptibility of Italian Aedes albopictus to Zika virus. Euro Surveill. 2016;21(18):30223.DOI: 10.2807/1560-7917.ES.2016.21.18.30223 PMID: 27171034
22. Ganushkina LA, Patraman IV, Rezza G, Migliorini L, Litvinov SK, Sergiev VP. Detection of Aedes aegypti, Aedes albopictus, and Aedes koreicus in the Area of Sochi, Russia.Vector Borne Zoonotic Dis. 2016;16(1):58-60.DOI: 10.1089/vbz.2014.1761 PMID: 26741323

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Autochthonous dengue outbreak in Nîmes, South of France, July to September 2015

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In August and September 2015, seven locally acquired cases of dengue virus type 1 (DENV-1) were detected in Nîmes, south of France, where *Aedes albopictus* has been established since 2011. Epidemiological and entomological investigations allowed to steer vector control measures to contain transmission. An imported case from French Polynesia with onset fever on 4 July was identified as primary case. This outbreak occurred from 8 August to 11 September in a 300 m radius area. Six sprayings to control mosquitos were performed in the affected area. We describe the first considerable dengue outbreak in mainland France where only sporadic cases of autochthonous dengue were recorded previously (2010, 2013 and 2014). The 69 day-period between the primary case and the last autochthonous case suggests multiple episodes of mosquito infections. The absence of notification of autochthonous cases during the month following the primary case's symptoms onset could be explained by the occurrence of inapparent illness. Recurrence of cases every year since 2013, the size of the 2015 outbreak and continuing expansion of areas with presence of *Ae. albopictus* highlight the threat of arboviral diseases in parts of Europe. Thus, European guidelines should be assessed and adjusted to the current context.

Background

Dengue viruses are usually transmitted in tropical areas by *Aedes* mosquitoes, primarily by *Aedes aegypti* and secondarily by *Ae. albopictus* [1]. In the European Basin, autochthonous transmission of dengue was not recorded from 1945 to 2010 mainly due to the disappearance of *Ae. aegypti* after the 1950s. The introduction of *Ae. aegypti* in 2005 in Madeira facilitated the occurrence of a large outbreak with more than 2,000 cases on the island in the Atlantic Ocean, in 2012 [2,3]. During the last four decades, *Ae. albopictus* has been reported in 20 European countries, mainly located in the Mediterranean basin [4]. Sporadic cases and pairs of cases of local dengue transmission have been reported in France in 2010, 2013 and 2014 [5-7]. In Croatia, an outbreak of dengue occurred in 2010 with laboratory evidence of recent infection in 17 people [8].

Following the establishment of *Ae. albopictus* in France in 2004, and given its potential as a vector of the dengue (DENV) and chikungunya (CHIKV) viruses, a national preparedness and response plan to prevent and control local transmission of chikungunya and dengue in mainland France was put in place in 2006 and has been updated every year since then [9]. It includes monitoring the geographical distribution of *Ae. albopictus* during the period of vector activity from May to November, and enhancing human surveillance – based on notification of suspected imported cases of dengue and/or chikungunya, and of confirmed autochthonous cases – in the districts where the vector is established.

The alert

On the 14 August 2015, two autochthonous cases of dengue were notified by the laboratory of virology of Nîmes University Hospital to the regional health authorities of Languedoc-Roussillon, an administrative region in the south of France. Real-time reverse transcriptase polymerisation chain reaction (RT-PCR) performed on samples taken 6 days after the onset of symptoms showed that both cases were positive for DENV. The two patients were around 20 years old and lived in the same house on the outskirts of Nîmes, in

the Gard administrative district (a subdivision of the administrative region of Languedoc-Roussillon). On 8 August 2015, they both developed a sudden high-grade fever (›38.5 °C) with headache, retro-orbital pain, myalgia, rash and asthenia. They had no travel history to dengue endemic or epidemic areas. Both were hospitalised from 13 to 17 August 2015. The French National Reference Centre (NRC) for arboviruses in Marseille, confirmed the diagnosis of dengue on 19 August by real-time RT-PCR, and characterised the virus serotype as DENV-1.

In line with the guidelines set down in the national preparedness and response plan, immediate epidemiological and entomological investigations were performed in order to contain transmission.

Methods

Epidemiological investigations

Imported dengue cases previously recorded in the surveillance database were retrospectively analysed to identify a potential primary case. All imported dengue cases identified during the 2015 season in Languedoc-Roussillon living near or visiting the same places where the two autochthonous cases lived, were listed.

For the investigation, the following case definitions of autochthonous dengue were applied. In the Gard administrative district as of 1 July 2015:

- A suspected case was defined by a sudden high-grade fever (›38.5 °C) associated with one of the following clinical signs (headache, myalgia, arthralgia, lower back pain, retro-orbital pain) which could not be explained by another medical condition, in a person with no history of travel to dengue endemic or epidemic areas in the 15 days before the onset of symptoms.

- A probable case was defined as a suspected case with an epidemiological link to a confirmed case (i.e. living in the same household as a confirmed imported or autochthonous case).

- A confirmed case was defined as a suspected case with positive laboratory tests (real-time RT-PCR or positive serology for IgM and IgG antibodies to DENV) performed by the NRC.

Active case finding was implemented on 20 August 2015 and included (i) door-to-door surveys in a 200 m radius around the index cases' residence, with neighbours being interviewed about any episodes of high temperature they may have had since 1 July, (ii) providing information and instruction to physicians and laboratories so that they could notify all suspected cases of dengue (imported and autochthonous) and (iii) phone calls to the 22 general practitioners working in a 1.5 km radius around the index cases' residence. Health authorities and epidemiologists interviewed all suspected, probable and confirmed dengue cases.
For each new suspected case identified, blood samples were collected for analysis by the NRC. Real-time RT-PCR was performed on samples collected within 7 days after symptoms onset. Serology was performed when samples were collected more than 5 days after the onset of symptoms.

Entomological investigation and vector control measures

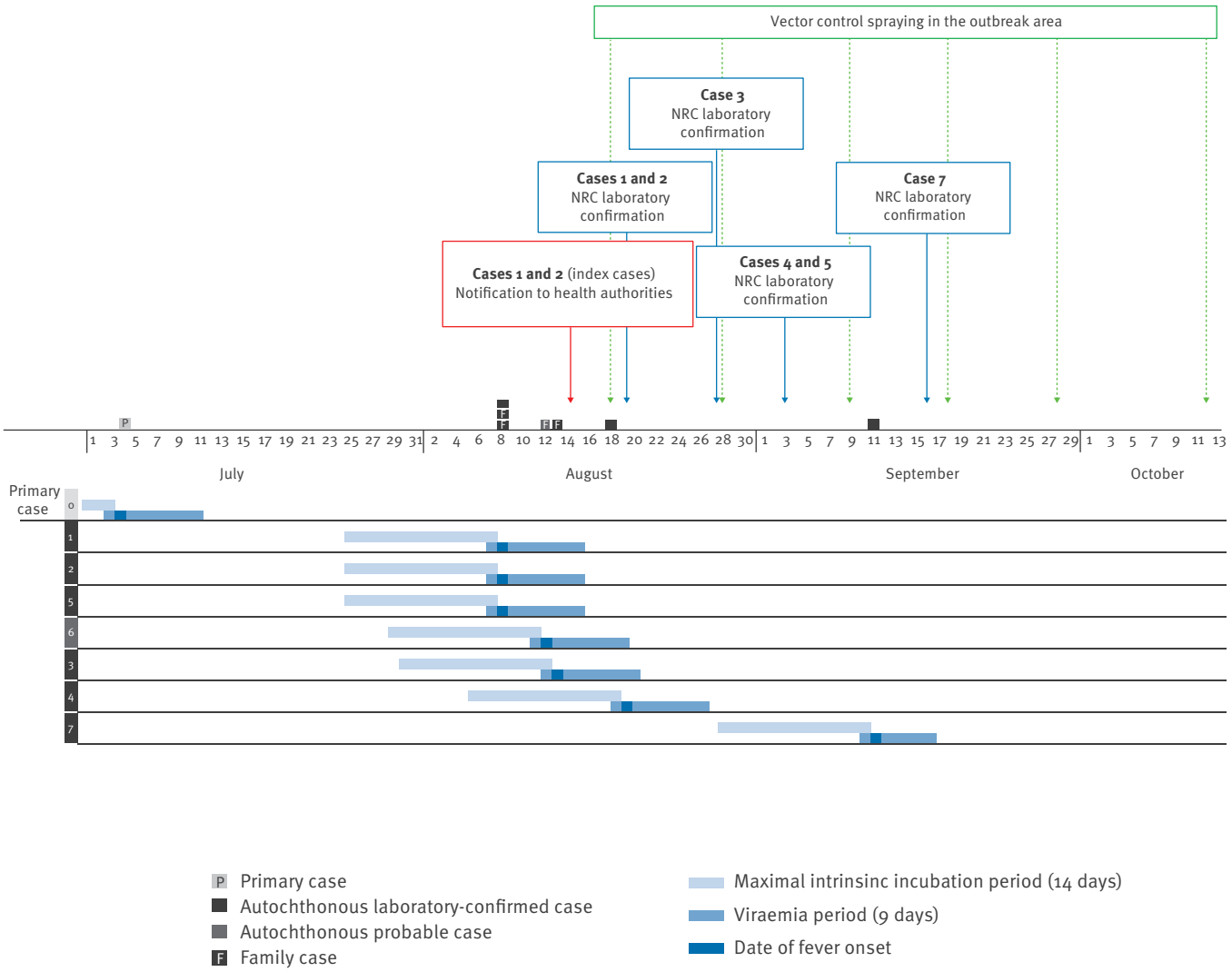
Entomological investigations were performed to guide vector control measures. Interviews focused on cases' (probable and confirmed) movements during their viraemic period (1 day before and 7 days after symptoms onset), including home, workplace, work-related travel, visits to friends and family and medical consultations. Control measures included the search for and elimination of possible larval breeding sites (by emptying water from containers or by using *Bti*), and ultra-low-volume spraying of deltamethrin (Cerathrine and Aqua K-Othrine 2 and 1g of active substance ha-1, respectively) in locations frequented by the cases during their viraemia when *Ae. albopictus* was found.

During the door-to-door survey, entomological data were also collected to estimate the number of possible breeding sites, larvae and imagos of *Ae. albopictus*. The house index, usually used to describe *Stegomyia* mosquito infestation of a city or district, was calculated. This index is the percentage of visited houses with at least one breeding site with larvae [10]. Due to lack of time, we did not calculate other indices, for example the Breteau index (the number of larvae-positive containers per 100 houses) or the container index (percentage of larvae-positive containers per 100 water-holding containers).

To assess DENV prevalence in the mosquito population in the area of autochthonous cases, a field survey with BG-Sentinel traps (with and without dry ice) was carried out twice. The first survey consisted of six traps positioned in the neighbourhood of autochthonous cases. The second survey covered a wider area (56.8 ha) and had 20 traps placed along the borders of the outbreak area. BG-traps were examined daily before and after deltamethrin treatment. Mosquito samples were transported in dry ice to the Maladies infectieuses et vecteurs: ecologie, génétique, evolution et contrôle (MIVEGEC) laboratory in Montpellier to be identified on a chill table and were stored at -80 °C to optimise the detection of RNA viruses. *Ae. albopictus* females were pooled in same day/location samples. Samples were then sent to the NRC to proceed with RNA extraction and qRT-PCR to detect possible DENV.

Figure 1

Timeline of symptoms onset for imported and autochthonous cases of dengue and epidemiological features, Nîmes, France, July–September 2015 (n = 8)



NRC: French National Reference Centre for arboviruses

Results

Epidemiological investigations

Primary case

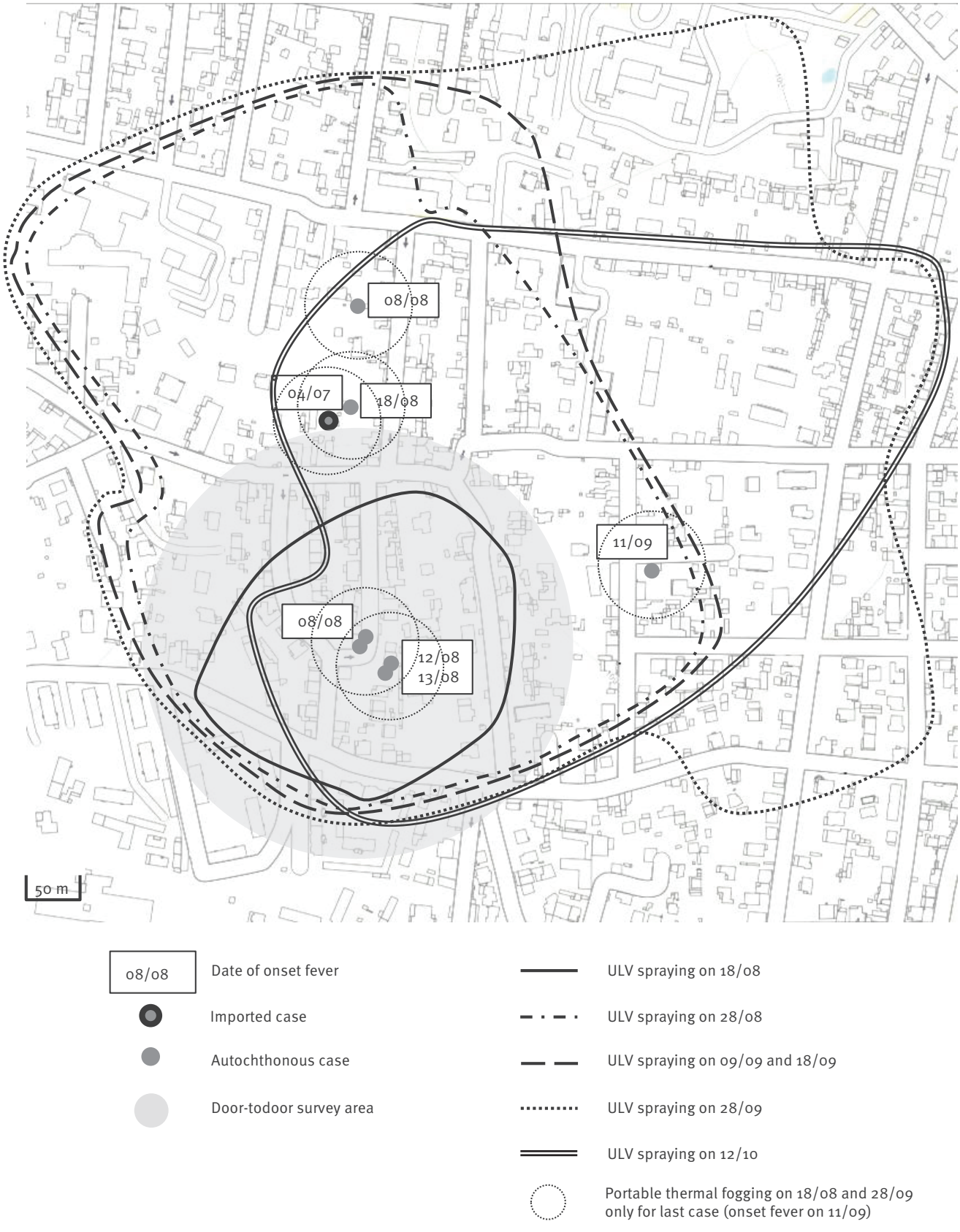
A dengue case imported from French Polynesia was identified in the surveillance database. The patient had developed sudden high fever with a headache, asthenia and diarrhoea on 4 July 2015, 5 days after returning from French Polynesia. The diagnosis was confirmed by real-time RT-PCR by the NRC and DENV-1 was identified. The case was notified to authorities on 14 July. Entomological investigations were performed and vector control measures subsequently implemented in the areas where the patient declared he had stayed during the viraemic phase from 3 to 11 July. The patient was interviewed once again in August after the identification of the two autochthonous cases. In this interview he also recalled that he had visited friends living in the outbreak neighbourhood during the viraemic phase. As he had not mentioned this location in his first interview, no vector control measures had been implemented in this neighbourhood in July.

Active case finding

Six suspected cases were identified through door-to-door surveys on 20 August. Of these, two agreed to provide a blood sample for virological investigations: one was positive following real-time RT-PCR, the other was negative. Among the four people who did not agree to provide a blood sample – for time and/or for personal reasons – one was classified as a probable case as he resided in the same household as a confirmed case (Table 1). After door-to-door surveys, three additional cases were confirmed by the NRC among suspected autochthonous cases notified by health professionals.

Figure 2

Spatial distribution of imported and autochthonous cases of dengue and vector control measures implemented, Nîmes, France, July–September (n=8)



ULV: ultra-low-volume.

Note: false-base map for privacy reasons.

Table 1

Final classification and laboratory results of dengue cases, Nîmes, France, July-September 2015 (n=8)

Case number	Classification	Date of symptoms onset	Sampling time after onset (days)	Serological tests for dengue		Real-time RT-PCR	Serotype	*Ct value*
				IgM	IgG			
Imported = primary case								
0	Confirmed	4 July 2015	3	ND	ND	Pos	DENV-1	24,09
Authochtonous case								
1[a]	Confirmed	8 Aug 2015	6	Pos	Neg	Pos	DENV-1	24,09
2[a]	Confirmed	8 Aug 2015	6	Pos	Neg	Pos	DENV-1	33,47
3	Confirmed	13 Aug 2015	3	ND	ND	Pos	DENV-1	20,77
4	Confirmed	18 Aug 2015	6	Pos	Pos	Pos	DENV-1	36,39
5	Confirmed[b]	8 Aug 2015	9	Pos	Neg	ND	ND	ND
6	Probable	12 Aug 2015	ND	ND	ND	ND	ND	ND
7	Confirmed	11 Sep 2015	1	ND	ND	Pos	DENV-1	15,52

Ct: cycle threshold; DENV: dengue virus; ND: Not done; Neg: negative; Pos: positive; RT: reverse-transcriptase.

[a]Index case.

[b]Increase of IgM and occurrence of IgG on a second sample performed 15 days after the first sample (antibodies seroconversion).

In total, seven autochthonous dengue cases where identified, six of whom were confirmed while one was probable (Table 1). Fever started from 8 August to 18 August for six cases, including the probable case, and on 11 September for one of the confirmed cases (Figure 1). All cases lived within a 300 m radius of the residence of the two index cases (Figure 2). The DENV-1 strain was identified for all five cases confirmed by real-time RT-PCR. The sex ratio was 1:1, the average age was 38 years old (mean: 38.6 years; range: 16-65). Clinical signs observed were a fever higher than 38.5 °C (8/8), headache (8/8), rash (5/8), retro-orbital pain (4/8), myalgia (4/8) and digestive disorders (5/8).

Entomological investigation and vector control measures

It is believed that the area where the cases were living has been colonised by *Ae. albopictus* since 2011, as it is located close to the places where *Ae. albopictus* populations were first detected in the city of Nîmes in that same year. The area, composed of small- and medium-sized houses with often interconnected gardens, and harboring many small breeding sites and rainwater collectors, is highly suited to the *Ae. albopictus*.

After interviewing cases on their movements during the viraemic phase, investigations were undertaken in 23 different sites in seven towns. The presence of vectors (larvae and/or imagos) was detected in 19 of these sites including the outbreak area.

Between 18 August and 12 October 2015, six mosquito control operations (spraying) were performed in the outbreak area (Figure 2). The first operation was performed in a 150 m radius around the residence of the two index cases. The spraying area was gradually expanded as new cases emerged. Sprayed surface areas ranged from 6.5 ha for the first operation, up to 52 ha for the final sixth operation. The first and second operations included houses of four cases. The other four operations included residences of all identified cases. In all cases except one, clinical signs occurred before or on the same day as the first vector control operations. For that one case clinical symptoms appeared after the third operation (Figure 1).

Investigations in other locations led to 18 mosquito control operations with chemical spraying. The average treated surface for each location was 5.7 ha.

During the door-to-door entomological investigation performed on 20 August, 91 houses were visited and 186 houses were not (151 absences and 35 refusals). A high abundance of *Ae. albopictus* at adult and larval stages was noted. The house index was 42% (38/91), and 20% (18/91) of houses were positive for imagos i.e. presence of at least one adult. The first survey to estimate the prevalence of dengue among mosquitoes lasted 24 hours and took place on 27 August, the day before the second control operation. The second survey lasted from 7 to 11 September, two days before and two days after the third control operation. We trapped 81 and 1,012 (219 males and 793 females) *Ae. albopictus*, respectively, in the two surveys. The presence of DENV was not detected in any of the 71 analysed pools of *Ae. albopictus*.

Discussion

Between 8 August and 11 September 2015, seven autochthonous cases of dengue were identified in the outskirts of Nîmes, a city of ca 150,000 inhabitants [11]. The outbreak, occurred within a 300 m radius around

the residence of an imported case, and the virus probably circulated for 3 months.

To date, this has been the largest dengue outbreak reported in mainland France, where only sporadic cases of autochthonous dengue had been reported previously [5-7]. The imported case who tested positive for DENV in July 2015 had returned from French Polynesia and often spent time in the affected district, especially during the viraemic phase. Identification of autochthonous cases of DENV-1 and the absence of other imported cases in the area are two strong arguments that this patient was indeed the primary case. The outbreak occurred in spite of the fact that the number of imported dengue cases was relatively low in 2015. Indeed, 12 imported dengue cases, with five in the Gard administrative district, were recorded in Languedoc-Roussillon region (which includes Gard) between 1 May and 30 November 2015, compared with 32 in 2013 and 24 in 2014 [12].

The outbreak area is quite a densely populated residential zone (55 persons per ha) with ca1,100 persons living in a habitat highly suited to *Ae. albopictus* [13]. Colonised by the vector for at least four years, vector density in the zone is high [14]. The episode investigated here follows a previous outbreak of chikungunya, which is transmitted by the same vector, in Montpellier city, in the Languedoc-Roussillon region in 2014. This confirms the threat of arboviral diseases transmitted by *Ae. albopictus* in the Mediterranean region [15].

Vector control measures (i.e. spraying with deltamethrin) had previously been implemented in the areas which the primary case mentioned he had visited and no local transmission of the virus was subsequently detected. However, forgetting to mention just one place in his initial interview was enough for the occurrence of autochthonous transmission in August 2015. This kind of monitoring failure, which is difficult to prevent and which will most likely happen again, highlights the importance of tracking data of the movements of imported cases. On the other hand, all but one case were infected before vector control measures started. The last case, which occurred after all six vector control operations had been implemented, was located on the margins of the treated area. Additional treatment was consequently implemented and no other case was later identified. Vector control measures were applied over a 150 m radius around the index cases' residence, in accordance with French national guidelines. However, three autochthonous cases and the imported case were located beyond this perimeter. Further work should focus on evaluating the effectiveness of control treatments, and whether control treatment areas should be widened.

Results for DENV detection in mosquitoes were negative. It must be noted, however, that most mosquito-trapping interventions were carried out after the first three mosquito control operations and over the whole investigated area. Recent studies on dengue transmission by *Ae. aegypti* [16] show a clear correlation between spatial proximity to dengue cases and infected mosquitoes. Therefore, all trapping interventions which aim to screen viruses in vectors should be focused on the immediate vicinity of cases and be implemented as precociously as possible. Considering an average life expectancy of 30 days for female *Ae. albopictus*, the 69 day-period between onset of symptoms in the primary case and the last autochthonous case suggests that several generations of mosquitoes were infected [17,18]. Furthermore, the absence of notification of autochthonous cases during the month following the onset of symptoms in the primary case could be explained by the occurrence of inapparent illness (ca 75% of cases in endemic territories) which may play an important role in the transmission chain [19,20]. In addition, we cannot exclude the possibility that symptomatic or pauci-symptomatic infections were not detected by the surveillance system. Serological investigation by public health services might lead to greater understanding and documentation of the dynamics of dengue transmission in Mediterranean urban contexts.

Conclusion

The epidemiology of arboviral diseases depends on multiple factors including environmental, behavioural and individual risk factors, as well as the effectiveness of vector control measures. Since its implementation in 2006, the French national preparedness and response plan to prevent and control local transmission of chikungunya and dengue has led to the detection and containment of several episodes of local transmission of dengue and chikungunya in the country. In the context of the continuing expansion of *Ae. albopictus* throughout parts of Europe, autochthonous cases of dengue are detected every year since 2013 with a larger outbreak in 2015 in France and an outbreak of chikungunya occurred in France in 2014 [15]. At a time when Zika virus is spreading in several countries in Central and South America, all highlight the growing risk of arboviral diseases transmitted by *Aedes* mosquitoes where this vector is established. New guidelines to prevent the spread of arboviral diseases in Europe should be developed and regularly assessed and adjusted to this evolving context. Health authorities need to continue and reinforce provision of information to the general population and travellers and to raise their awareness for the transmission and prevention of arboviral diseases in regions where the vector is established.

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Conflict of interest

None declared

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References

1. Gubler DJ. Dengue, Urbanization and Globalization: The Unholy Trinity of the 21(st) Century.Trop Med Health. 2011;39(4Suppl):3-11.DOI: 10.2149/tmh.2011-S05 PMID: 22500131
2. Schaffner F, Mathis A. Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. Lancet Infect Dis. 2014;14(12):1271-80.DOI: 10.1016/S1473-3099(14)70834-5 PMID: 25172160
3. Wilder-Smith A, Quam M, Sessions O, Rocklov J, Liu-Helmersson J, Franco L, et al. The 2012 dengue outbreak in Madeira: exploring the origins. Euro Surveill. 2014;19(8):20718.DOI: 10.2807/1560-7917.ES2014.19.8.20718 PMID: 24602277
4. Medlock JM, Hansford KM, Schaffner F, Versteirt V, Hendrickx G, Zeller H, et al. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector Borne Zoonotic Dis. 2012;12(6):435-47.DOI: 10.1089/vbz.2011.0814 PMID: 22448724
5. La Ruche G, Souarès Y, Armengaud A, Peloux-Petiot F, Delaunay P, Desprès P, et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveill. 2010;15(39):19676.PMID: 20929659
6. Marchand E, Prat C, Jeannin C, Lafont E, Bergmann T, Flusin O, et al. Autochthonous case of dengue in France, October 2013. Euro Surveill. 2013;18(50):20661.DOI: 10.2807/1560-7917.ES2013.18.50.20661 PMID: 24342514
7. Giron S, Rizzi J, Leparc-Goffart I, Septfons A, Tine R, Cadiou B, et al. New occurrence of autochthonous cases of dengue fever in Southeast France, August-September 2014. Bull Epidemiol Hebd (Paris). 2015; (13-14):217-23.
8. Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobučar A, Pem-Novosel I, et al. Autochthonous dengue fever in Croatia, August-September 2010. Euro Surveill. 2011;16(9):19805.PMID: 21392489
9. Ministère des affaires sociales, de la santé et des droits des femmes. [Ministry of Social Affairs, health and women's rights.]. Instruction N. °DGS/RI1/2015/125 du 16 avril 2015 mettant à jour le guide relative aux modalités de mise en œuvre du plan anti-dissémination du chikungunya et de la dengue en métropole. [Instruction N ° DGS / RI1 / 2015/125 of 16 April 2015 updating the guide on the implementation modalities of the national preparedness and response plan to prevent and control local transmission of chikungunya and dengue.] Paris: Ministère des affaires sociales, de la santé et des droits des femmes; 2015.
10. Service MW. Mosquito ecology: field sampling methods. 2nd ed. London: Chapman et Hall;1993.
11. Institut national de la statistique et des études économiques (Insee). [National Institute of Statistics and Economic Studies (Insee)]. Populations légales du département du Gard, ses arrondissements, ses cantons et ses communes. [Official population of the Gard district, its boroughs, cantons and communes]. Institut nationale de la statistique et des études économiques. Paris: Insee; 2015 (cited 22 Oct 2015). Available from: http://www.insee.fr/fr/ppp/bases-de-donnees/recensement/populations-legales/departement.asp?dep=30&annee=2012
12. Santé publique France. Chikungunya et dengue - Données de la surveillance renforcée en France métropolitaine. [Chikungunya and dengue - enhancing human surveillance in France]. Updated 5 May 2015, cited 24 May 2015. Saint-Maurice: Santé publique France. Available from: http://www.invs.sante.fr/Dossiers-thematiques/Maladies-infectieuses/Maladies-a-transmission-vectorielle/Chikungunya/Donnees-epidemiologiques.
13. Jourdain F, Roiz D, Perrin Y, Grucker K, Simard F, Paupy C. [Entomological factors of arboviruses emergences]. Transfus Clin Biol. 2015;22(3):101-6.DOI: 10.1016/j.tracli.2015.06.001 PMID: 26141429
14. Le Préfet du Gard. [Prefecture of the Gard district]. Arrêté préfectoral n°2011279-002 du 6 octobre 2011 relatif aux modalities de mise en oeuvre du plan anti-dissémination du chikungunya et de la dengue dans le département du Gard. [Prefectural order No. 2011279-002 of 6 October 2011 on the Modalities of the national preparedness and response plan to prevent and control local transmission of chikungunya and dengue]. 6 Oct 2011. Available from: http://www.gard.gouv.fr/content/download/2416/17437/file/RAA%202011-10-B%20publi%C3%A9%20le%206%20OCTOBRE%20%202011.pdf
15. Delisle E, Rousseau C, Broche B, Leparc-Goffart I, L'Ambert G, Cochet A, et al. Chikungunya outbreak in Montpellier, France, September to October 2014. Euro Surveill. 2015;20(17):21108. DOI: 10.2807/1560-7917.ES2015.20.17.21108 PMID: 25955774
16. Thomas SJ, Aldstadt J, Jarman RG, Buddhari D, Yoon IK, Richardson JH, et al. Improving dengue virus capture rates in humans and vectors in Kamphaeng Phet Province, Thailand, using an enhanced spatiotemporal surveillance strategy. Am J Trop Med Hyg. 2015;93(1):24-32.DOI: 10.4269/ajtmh.14-0242 PMID: 25986580
17. Chan M, Johansson MA. The incubation periods of Dengue viruses.PLoS One. 2012;7(11):e50972.DOI: 10.1371/journal.pone.0050972 PMID: 23226436
18. Delatte H, Gimonneau G, Triboire A, Fontenille D. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of Aedes albopictus, vector of chikungunya and dengue in the Indian Ocean.J Med Entomol. 2009;46(1):33-41.DOI: 10.1603/033.046.0105 PMID: 19198515
19. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504-7.DOI: 10.1038/nature12060 PMID: 23563266
20. Duong V, Lambrechts L, Paul R.E, Ly S, Srey Lay R, Long K.C, et al. Asymptomatic humans transmit dengue virus to mosquitoes. Proc Natl Acad Sci U S A. 2015;112(47):14688-93.

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Serotype/serogroup-specific antibiotic non-susceptibility of invasive and non-invasive *Streptococcus pneumoniae*, Switzerland, 2004 to 2014

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Concurrent analysis of antibiotic resistance of colonising and invasive *Streptococcus pneumoniae* gives a more accurate picture than looking at either of them separately. Therefore, we analysed 2,129 non-invasive and 10,996 invasive pneumococcal isolates from Switzerland from 2004 to 2014, which spans the time before and after the introduction of the heptavalent (PCV7) and 13-valent (PCV13) conjugated pneumococcal polysaccharide vaccines. Serotype/serogroup information was linked with all antibiotic resistance profiles. During the study period, the proportion of non-susceptible non-invasive and invasive isolates significantly decreased for penicillin, ceftriaxone, erythromycin and trimethoprim/sulfamethoxazole (TMP-SMX). This was most apparent in non-invasive isolates from study subjects younger than five years (penicillin ($p=0.006$), erythromycin ($p=0.01$) and TMP-SMX ($p=0.002$)). Resistant serotypes/serogroups included in PCV7 and/or PCV13 decreased and were replaced by non-PCV13 serotypes (6C and 15B/C). Serotype/serogroup-specific antibiotic resistance rates were comparable between invasive and non-invasive isolates. Adjusted odds ratios of serotype/serogroup-specific penicillin resistance were significantly higher in the west of Switzerland for serotype 6B (1.8; 95% confidence interval (CI): 1.4–4.8), 9V (3.4; 95% CI: 2.0–5.7), 14 (5.3; 95% CI: 3.8–7.5), 19A (2.2; 95% CI: 1.6–3.1) and 19F (3.1; 95% CI: 2.1–4.6), probably due to variations in the antibiotic consumption.

Introduction

Antibiotic resistance in *Streptococcus pneumoniae* is a worldwide concern and can lead to treatment failures with increase in morbidity and mortality, augmented treatment cost and use of more toxic reserve antimicrobials [1,2]. In this context, surveillance of antibiotic resistance in *S. pneumoniae* is important for the appropriate choice of empirical therapy, to detect new resistance developments in a timely manner and to monitor the effect of interventions such as antibiotic stewardship campaigns or vaccines on resistance rates and serotype distribution [3].

Heptavalent conjugated pneumococcal polysaccharide vaccine (PCV7) followed by 13-valent vaccine (PCV13) have been recommended and reimbursed by the health insurance in Switzerland since late 2006 and 2011, respectively, for all children younger than two years [4]. During the period from 2008 to 2010, the pneumococcal vaccine coverage was, respectively, ca 50% (95% confidence interval (CI): 46.0–53.5) and 37% (95% CI: 32.5–41.6) for two and three doses at the age of two years [4]. The coverage increased to 79% (95% CI: 77.4–80.4) and 75% (95% CI: 73.8–76.7), respectively, for two and three doses between 2011 and 2013. Introduction of vaccines led to a decrease in the incidence in invasive pneumococcal disease (IPD) but also to a change in serotype redistribution across all ages [4]. In other countries, vaccine introduction has additionally led to a decrease in antibiotic resistance rates because the less susceptible serotypes have been included in the vaccines [5]. So far, in Switzerland, antibiotic resistance in *S. pneumoniae* has only been analysed in the pre-PCV7 era from 2001 to 2004 [6].

Ideally, antibiotic resistance rates in non-invasive and invasive *S. pneumoniae* are analysed simultaneously, but such studies of representative size are rare or often not very recent [6,7] because in many countries, antibiotic resistance data is exclusively drawn from surveillance of invasive isolates. Switzerland runs two different national surveillance systems collecting resistance data on *S. pneumoniae:* sentinel surveillance

Figure 1

Proportions of non-susceptibility of *Streptococcus pneumoniae* isolates, Switzerland, 2004–14 (n = 13,125)

IPD: invasive pneumococcal disease; TMP-SMX: trimethoprim/sulfamethoxazole.

Antibiotic resistance towards penicillin includes isolates with MIC › 0.06 mg/L

Samples for which age group was not known (n = 681) are excluded from this analysis.

Analyses were stratified according to IPD in patients‹5 years (A), 5–64 years (B),›64 years of age (C) and non-IPD in patients‹5 years of age (D). Data on non-IPD in patients 5–64 years and›64 years of age were omitted due to low number of isolates in these two categories.

of outpatient non-invasive pneumococci (Sentinella) and comprehensive passive surveillance of all invasive pneumococci [4,8]. The aims of this study were (i) to simultaneously describe the prevalence of antibiotic resistance in invasive and non-invasive *S. pneumoniae* in different patient populations in Switzerland from 2004 to 2014, (ii) to analyse possible temporal trends and effects of PCV7 and PCV13 on resistance prevalence, (iii) to detect serotype/serogroup-specific antibiotic resistance and (iv) to analyse regional differences for the antibiotic resistance rates.

Methods

Sentinel surveillance of non-invasive pneumococcal isolates (Sentinella)

Between 2004 and 2014, data on colonising pneumococci were obtained from a nationwide, ongoing, prospective surveillance study within the Swiss Sentinel System which has been described in detail previously [8]. In brief, this network involves a chosen sample of practitioners who represent Switzerland geographically and demographically. The overall number of participants per subspecialty in the Sentinel System is defined as a proportion of all Swiss practitioners in the matching specialty [8,9]. Therefore, ca 200 practitioners (general practitioners, internists and paediatricians) took samples of outpatients who were clinically diagnosed with acute otitis media or pneumonia [8]. All received swabs were cultured for *S. pneumoniae* at the Swiss National Reference Centre for Pneumococci (NZPn) as described [6].

Comprehensive surveillance of invasive pneumococcal isolates

Physician reporting of invasive pneumococcal infection has been mandatory in Switzerland since 1999 (http://www.bag.admin.ch). In March 2002, the NZPn was set up and has since been prospectively collecting clinical pneumococcal isolates from normally sterile body sites (blood, cerebrospinal, joint, pleural and peritoneal fluid but not middle ear fluid) sent in by Swiss clinical microbiology laboratories. It is possible to link ca 90% of physician-reported IPD cases with a corresponding pneumococcal isolate, indicating a very high participation of involved laboratories and a high completeness of data linkage [4]. This study uses only the NPZn isolates and demographic data associated with them. Physician-reported data are not included in the analysis in this study.

Analysis of non-invasive and invasive isolates

All isolates were confirmed as *S. pneumoniae* by alpha haemolysis morphology on blood agar plates, bile solubility and optochin sensitivity. Serotypes of all confirmed pneumococcal isolates were determined by the Quellung reaction.

Methods for antibiotic resistance testing were identical for non-invasive and invasive isolates and have been described previously [8]. In brief, all isolates were tested against oxacillin (1 µg disk), erythromycin, trimethoprim/sulfamethoxazole (TMP-SMX) and levofloxacin by the disk diffusion method. For isolates with reduced susceptibility to oxacillin, the minimum inhibitory concentration (MIC) against penicillin and ceftriaxone was determined by Etest (AB Biodisk) according to Clinical and Laboratory Standards Institute (CLSI) [10]. During the study period, CLSI introduced new MIC breakpoints for parenteral penicillin therapy of non-meningitis infections. To ensure that the same breakpoints were applied for all isolates over the entire study period, we used the oral non-meningitis breakpoints (susceptible (S) ≤ 0.06 mg/L or oxacillin disk diameter ≥ 20 mm) for penicillin non-susceptibility and the meningitis (S ≤ 0.5 mg/L) breakpoints for ceftriaxone non-susceptibility for the entire time period.

For this study, data for isolates collected between January 2004 and December 2014 were analysed. Data on patients' age, sex and geographical origin of samples were available for all isolates except 681 records with missing data on age and 6 records with missing data on geographical region.

Statistical analysis

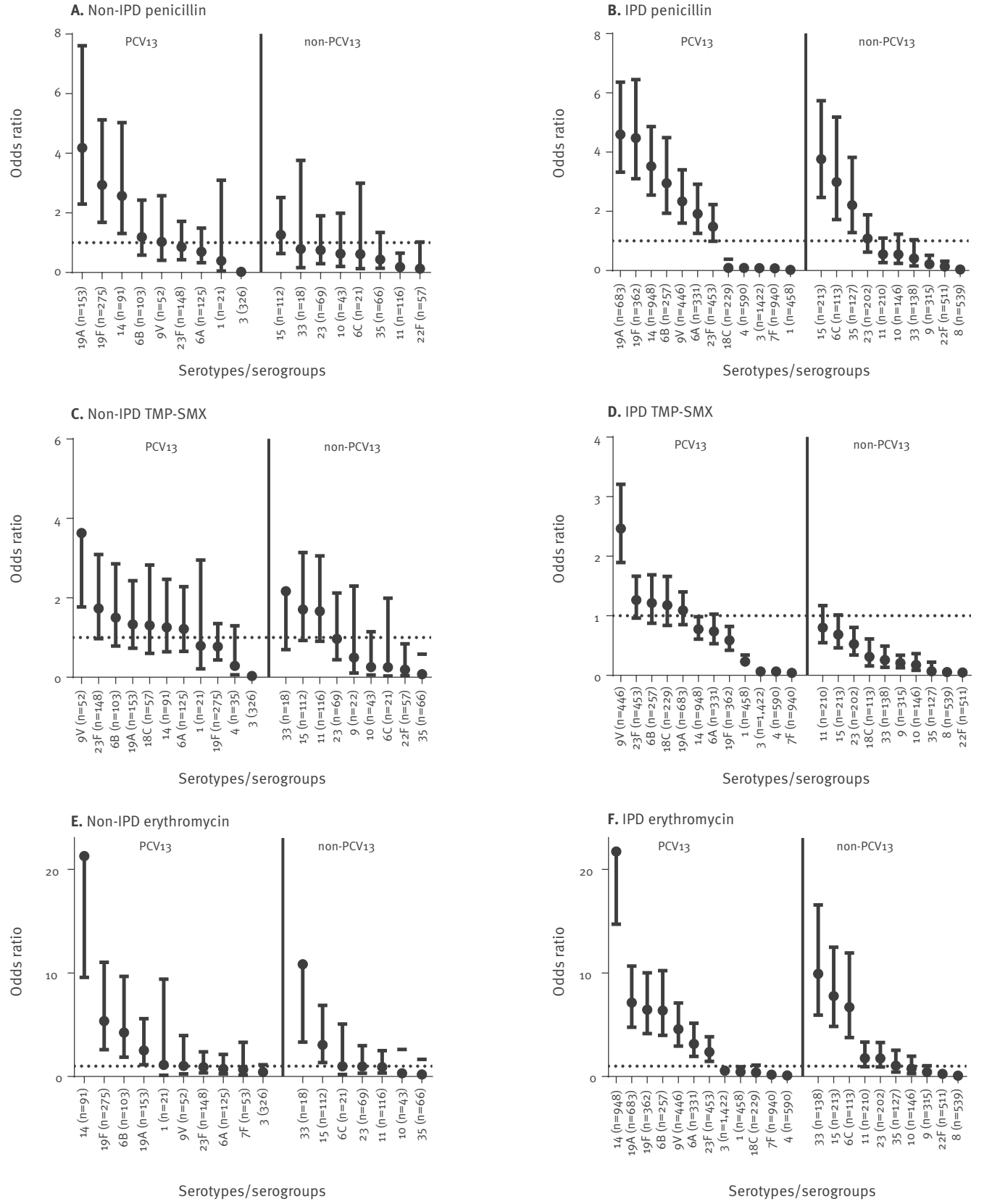
Resistance prevalence data were stratified by age group (‹5, 5–64 and›64 years), serotype/serogroup and geographical region. For geographical comparisons, Switzerland was divided into two regions, the French speaking western part designated 'west' and the remaining parts of the country including the Italian speaking canton Ticino designated 'other', as described previously [6]. Differences were calculated using 2 × 2 or 3 × 2 chi-square test. Changes over time from 2004 to 2014 were analysed with the chi-square test for trend with CDC EpiInfo Version 7 and GraphPad Prism version 6.00 for Windows, GraphPad Software. A cut-off value of $p \leq 0.05$ (two-tailed) was used for these tests.

Serotype/serogroup-specific antibiotic non-susceptibilities of pencillin, erythormycin or TMP-SMX of invasive and colonising *S. pneumoniae* isolates were calculated by multivariate logistic regression analysis. Adjusted odds ratios (aOR) with 95% confidence intervals (95% CI) were received for serotypes/serogroups with overall proportions of above 1%. For all six regression analyses, the remaining serotypes, consisting of serotypes/serogroups with overall proportions below 1% in invasive and non-invasive *S. pneumoniae*, served as the reference group.

Serotype/serogroup-specific antibiotic resistance by geographical region and isolation site (i.e. colonising vs invasive *S. pneumoniae*) for the top four resistant serotypes for penicillin, erythromycin and trimethoprim/sulfamethoxazole (TMP-SMX) was also calculated by multivariate logistic regression analysis. Isolates from the 'other' part of Switzerland were used as the reference group for each of the 12 regression analyses.

Figure 2

Serotype/serogroup-specific multivariate logistic regression analysis, *Streptococcus pneumoniae* isolates, Switzerland, 2004–14 (n = 12,438)



IPD: invasive pneumococcal disease; PCV: pneumococcal conjugated vaccine; TMP-SMX: trimethoprim/sulfamethoxazole.

Analysis of serotype/serogroups with penicillin non-susceptibility in non-invasive (A) and invasive (B) samples. TMP-SMX non-susceptibility is illustrated for non-invasive (C) and invasive (D) samples. Erythromycin non-susceptibility is shown for non-invasive (E) and invasive (F) samples. Odds ratios were adjusted for year of isolation, geographical region (west vs other) and age category (<5; 5–64 and>64 years). Serotypes/serogroups with proportion of<1% were combined to one group for the analysis and served as reference group. In the reference group, there were 680 invasive and 148 colonising isolates, respectively. PCV13 serotypes are indicated. PCV7 serotypes are included in the PCV13 serotypes and are 4, 6B, 9V, 14, 18C, 19F and 23F.

Isolates with unknown age (n = 681) and/or geographical region known (n = 6) were excluded.

Table 1

Characteristics of invasive (n = 10,996) and non-invasive (n = 2,129) pneumococcal isolates, Switzerland, 2004–14

	Non-invasive *Streptococcus pneumoniae*		Invasive *Streptococcus pneumoniae*		
	n	%	n	%	p[a]
Total isolates	2,129	100.0	10,996	100.0	NA
Age					
< 5 years	1,347	63.3	657	6.0	<0.001
5–64 years	710	33.3	4,164	37.9	<0.001
> 64 years	70	3.3	5,496	50.0	<0.001
Not known	2	0.1	679	6.2	<0.001
Region					
West	856	40.2	2,781	25.3	<0.001
Other	1,271	59.7	8,211	74.7	<0.001
Not known	2	0.1	4	0.0	0.3
Non-susceptibility					
Penicillin[b]	299	14.0	1,077	9.8	<0.0001
Ceftriaxone	35	1.6	242	2.2	0.04
Erythromycin	256	12.0	1,295	11.8	0.7
TMP-SMX	328	15.4	1,540	14.0	0.09
Levofloxacin	1	0.0	41	0.4	0.02
Serotype/serogroup [c]					
Age < 5 years	n = 1,347		n = 657		
PCV7	532	39.5	242	36.8	0.3
PCV13 minus PCV7	379	28.1	280	42.6	<0.0001
Non-PCV13	436	32.4	135	20.4	<0.0001
Age 5–64 years	n = 710		n = 4,164		
PCV7	212	29.9	1,228	29.5	0.8
PCV13 minus PCV7	283	39.9	1,667	40.0	0.9
Non-PCV13	215	30.3	1,269	30.5	0.9
Age > 64 years	n = 70		n = 5,495		
PCV7	17	24.3	1,816	33.0	0.1
PCV13 minus PCV7	18	25.7	1,911	34.8	0.1
Non-PCV13	35	50.0	1,768	31.2	0.002

NA: not applicable; TMP-SMX: trimethoprim/sulfamethoxazole.

[a] p value calculated with 2 × 2 chi-square test.

[b] Minimal inhibitor concentration > 0.06 mg/L.

[c] PCV7: serotypes/serogroups 4, 6B, 9V, 14, 18C, 19F, 23F; PCV13 minus PCV7: serotypes/serogroups 1, 3, 5, 6A, 7F, 19A; PCV13: serotypes/serogroups 4, 6B, 9V, 14, 18C, 19F, 23F, 1, 3, 5, 6A, 7F, 19A; Non-PCV13: all remaining serotypes/serogroups. For two and 679 non-invasive and invasive isolates, respectively, age was unknown. For one invasive isolate, the serotype/serogroup was unknown.

Logistic regression analyses were performed using Stata (version 13.0).

Results

In total, 2,129 non-invasive and 10,996 invasive pneumococcal isolates from January 2004 to December 2014 were analysed (Table 1). The patient population with non-invasive *S. pneumoniae* had a significantly higher proportion of children younger than five years than the patient population with invasive isolates (Table 1; p<0.001). This is because non-invasive isolates were received from patients with pneumonia (n=285), but more often from patients with acute otitis media (n=1,775) who are mostly toddlers. For 69 isolates, the underlying disease was unknown. In general, isolates from the west were overrepresented among the non-invasive isolates and proportional to the resident population among the invasive isolates (Table 1).

Overall, we identified 22 serotypes/serogroups with a prevalence of more than 1.0% (serotypes/serogroups 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9, 9V, 10, 11, 14, 15, 18C, 19A, 19F, 22F, 23, 23F, 33 and 35). The proportions of isolates with PCV7 serotypes did not differ between the non-invasive and the invasive isolates in any of the three chosen age categories (<5 years, 5–64 years and >64 years). In contrast, differences were noted for the PCV13 minus PCV7 (serotypes/serogroups 1, 3, 5, 6A, 7F, 19A) and non-PCV13 serotypes/serogroups

Table 2

Antibiotic-resistant pneumococcal isolates by age and geographical origin of samples, Switzerland, 2004–14 (n = 13,125)

	‹5 years		5–64 years		›64 years		P[a]	West[b]		Other[c]		P[d]
	n	%	n	%	n	%		n	%	n	%	
Resistance Invasive												
Penicillin (MIC › 0.06 mg/L)	114	17.4	392	9.4	483	8.8	‹0.0001	449	16.1	628	7.6	‹0.0001
Penicillin (MIC › 2.0 mg/L)	10	1.5	32	0.8	28	0.5	0.008	30	1.1	45	0.5	0.003
Ceftriaxone[e]	33	5.0	86	2.1	98	1.8	‹0.0001	121	4.4	121	1.5	‹0.0001
Erythromycin[e]	125	19.0	440	10.6	646	11.8	‹0.0001	425	15.3	870	10.6	‹0.0001
TMP-SMX[e]	124	18.9	601	14.4	693	12.6	‹0.0001	479	17.2	1059	12.9	‹0.0001
Levofloxacin[e]	1	0.2	8	0.2	29	0.5	0.02	10	0.4	31	0.4	0.9
Total[f]	**657**	**100.0**	**4,164**	**100.0**	**5,496**	**100.0**	**NA**	**2,781**	**100.0**	**8,211**	**100.0**	**NA**
Resistance non-invasive												
Penicillin (MIC › 0.06 mg/L)	226	16.8	70	9.9	3	4.3	‹0.0001	175	20.4	124	9.8	‹0.0001
Penicillin (MIC › 2.0 mg/L)	9	0.7	3	0.4	1	1.4	0.5	9	1.1	4	0.3	0.03
Ceftriaxone[e]	25	1.9	9	1.3	1	1.4	0.6	25	2.9	10	0.8	‹0.0001
Erythromycin[e]	205	15.2	4	6.6	4	5.7	‹0.0001	151	17.6	105	8.3	‹0.0001
TMP-SMX[e]	227	16.9	92	13.0	9	12.9	0.06	150	17.5	178	14.0	0.03
Levofloxacin[e]	0	0	1	0.1	0	0	0.4	0	0	1	0.1	0.4
Total[f]	**1,347**	**100.0**	**710**	**100.0**	**70**	**100.0**	**NA**	**856**	**100.0**	**1,271**	**100.0**	**NA**

MIC: minimum inhibitory concentration; NA: not applicable.

Samples for which age group (n = 681) and/or geographical origin (n = 6) were not known are excluded from the respective analyses.

[a] p value calculated with 3 × 2 chi-square test.

[b] Includes the French-speaking and bilingual French- and German-speaking Swiss Cantons.

[c] Includes the German- and Italian-speaking Cantons.

[d] p value calculated with 2 × 2 chi-square test.

[e] Includes isolates with intermediate (i) and full antibiotic resistance (r).

[f] Total number of isolates tested for antibiotic resistance. Susceptible isolates are not shown, but included in the total.

within individuals younger than five years and older than 64 years (Table 1).

Antibiotic resistance rates were highest in children younger than five years, for both invasive and non-invasive isolates, for all tested antibiotics (penicillin, ceftriaxone, erythromycin and TMP-SMX) with the exception of levofloxacin (Table 2). In general, antibiotic resistance rates were comparable between invasive and non-invasive isolates in all age groups. Non-susceptibility was higher in western Switzerland for penicillin, ceftriaxone, erythromycin and TMP-SMX for invasive as well as non-invasive isolates (Table 2).

Time trends of pneumococcal antibiotic resistance prevalence

We subsequently analysed the temporal evolution of antibiotic non-susceptibility rates for three different age groups (Table 3 and Figure 1). Chi-square test for trend over the individual years 2004 to 2014 showed significant decreasing trends for penicillin, erythromycin and TMP-SMX non-susceptibility in patients five to 64 years of age with invasive isolates. The same was true for the same three antibiotics in children younger than five years with non-invasive pneumococci. Further significant downward trends of non-susceptibility were seen in invasive isolates for erythromycin in the under five-year-olds and for ceftriaxone and TMP-SMX in the elderly (›64 years). The lowest non-susceptibility rates overall were found in the most recent years (2013–14), representing three and eight years, respectively, after the PCV13 and PCV7 vaccination recommendations for children under the age of two years.

Serotype/serogroup-specific antibiotic resistance was independent of the site of isolation

Figure 2 shows serotype/serogroup-specific antibiotic resistance for the three antibiotics penicillin, erythromycin and TMP-SMX. Results for ceftriaxone and levofloxacin were omitted as the numbers were low. The aORs were calculated for 22 serotypes/serogroups with individual proportions of above 1% overall (non-invasive and invasive isolates pooled). The serotypes consisting of serotypes/serogroups with overall individual proportions below 1% served as the reference group. Overall, ranking order of aORs of being non-susceptible revealed remarkable similarities between non-invasive

Table 3

Time trends of non-susceptibility of pneumococcal isolates by year[a], Switzerland, 2004–14 (n = 13,125)

Age (years)	Non-susceptibility	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total	Trend[b]
Invasive *S. pneumoniae*														
< 5	Penicillin	11	14	12	17	12	11	12	8	9	1	7	114	1
	Ceftriaxone	2	7	3	6	4	4	2	1	3	0	1	33	0.2
	Erythromycin	18	23	11	15	20	6	9	11	5	3	4	125	0.008
	TMP-SMX	20	14	16	12	15	13	9	7	8	3	7	124	0.2
5–64	Penicillin	43	48	28	31	42	46	45	28	37	25	19	392	0.009
	Ceftriaxone	5	15	2	16	7	11	12	4	5	7	2	86	0.08
	Erythromycin	46	39	41	49	46	66	40	29	37	29	18	440	0.0005
	TMP-SMX	82	76	39	63	72	53	46	39	40	52	39	601	**<0.0001**
> 64	Penicillin	29	43	48	48	45	47	68	47	46	34	28	483	0.4
	Ceftriaxone	3	13	9	20	7	13	15	4	8	5	1	98	0.01
	Erythromycin	44	50	65	84	76	61	53	56	57	51	49	646	0.1
	TMP-SMX	80	88	53	69	68	68	64	48	68	44	43	693	**<0.0001**
Total[c]		**855**	**943**	**874**	**998**	**1,076**	**1,082**	**950**	**974**	**852**	**926**	**787**	NA	
Non-invasive *S. pneumoniae*														
< 5	Penicillin	44	46	44	24	15	13	18	16	6	3	1	230	0.006
	Ceftriaxone	3	4	3	5	3	4	3	0	0	0	0	25	0.6
	Erythromycin	38	43	38	24	12	10	13	13	8	3	3	205	0.01
	TMP-SMX	52	50	33	24	14	7	15	14	6	7	5	227	0.002
5–64	Penicillin	14	9	10	8	5	10	4	1	3	5	1	70	0.4
	Ceftriaxone	2	0	2	2	2	1	0	0	0	0	0	9	0.3
	Erythromycin	7	7	6	4	2	7	5	0	5	4	0	47	0.6
	TMP-SMX	8	26	14	7	10	7	5	3	6	2	4	92	0.4
> 64	Penicillin	1	0	1	1	0	0	0	0	0	0	0	3	0.08
	Ceftriaxone	0	0	1	0	0	0	0	0	0	0	0	1	0.4
	Erythromycin	0	0	1	0	1	0	0	1	1	0	0	4	0.8
	TMP-SMX	2	1	1	1	1	1	0	0	1	0	1	9	0.2
Total[c]		**340**	**344**	**354**	**193**	**181**	**182**	**137**	**147**	**118**	**70**	**61**	**NA**	

NA: not applicable; TMP-SMX: trimethoprim/sulfamethoxazole.

Samples for which age group was not known (n = 681) are excluded from this analysis.

[a] White shading: pre-PCV7 years; light grey shading: post-PCV7 but pre-PCV13 years; dark grey shading: post-PCV13 years.

[b] p value calculated by chi-square test for trend. Significant p values indicated in bold (i.e. p < 0.05).

[c] Total number of isolates tested in all three age groups combined for which patient age was known (total invasive isolates: n = 10,317; total non-invasive isolates: n = 2,127). Susceptible isolates are not shown, but included in the total.

and invasive *S. pneumoinae* isolates. The four serotypes with lowest susceptibility and therefore with the highest aORs for penicillin were identical in the groups of non-invasive and invasive isolates (19A, 19F, 14 and 15; Figures 2A and B). For TMP-SMX, the order of aORs was different between invasive and non-invasive isolates, but the serotype with the lowest susceptibility (serotype 9V) was again identical (Figures 2C and D). Finally, serogroups 14 and 33 had the highest aORs for erythromycin non-susceptibility in both collections (Figures 2E and F). Thus, a distinct, serotype-specific antibiotic resistance exists and is likely to be independent of the anatomical isolation site (Figures 2A-2F).

Most serotypes revealing significantly higher antibiotic resistance rates than the reference group were covered by PCV13 (Figures 2A-2F), and indeed, introduction of the vaccine mitigated the prevalence of pneumococcal antibiotic resistance (Table 3). However, non-PCV13 serogroups/serotypes 15, 6C and 33 were also associated with increased antibiotic resistance to penicillin and/or erythromycin.

Serotype/serogroup distribution by geographical region

We calculated the serotype/serogroup-specific antibiotic resistance of the four most resistant *S. pneumoniae* serotypes for the three antibiotics penicillin, TMP-SMX and erythromycin by geographical region in Switzerland (west vs other; Table 4). We found that the geographical region had a profound effect on serotype/serogroup-specific antibiotic resistance (Table 4). In order to determine if these differences were significant, we performed a logistic regression for each

Table 4

Serotype/serogroup-specific penicillin resistance of invasive and colonising *Streptococcus pneumoniae* isolates, by geographical region, Switzerland, 2004–14 (n = 13,125)

Serotype/ serogoup	Number of isolates[b]	Antibiotic	% SSR			Adjusted OR (95% CI) for West[a]
			All	West	Other	
19A	884	Penicillin	32.4	44.9	25.9	2.2 (1.6–3.1)
19F	663		30.3	46.5	22.7	3.0 (2.0–4.4)
14	1,095		24.7	50.8	16.2	5.3 (3.8–7.4)
15	338		23.7	26.9	21.9	1.6 (0.9–2.9)
9V	526	TMP-SMX	44.9	58.0	40.2	2.1 (1.4–3.3)
23F	632		29.1	34.8	26.8	1.6 (1.1–2.5)
6B	374		27.8	28.6	27.5	1.0 (0.6–1.8)
18C	304		27.6	30.1	26.7	1.2 (0.6–2.2)
14	1,095	Erythromycin	49.3	61.9	45.2	1.8 (1.4–2.5)
33	160		33.1	54.4	24.6	3.7 (1.5–9.5)
19A	884		24.9	33.2	20.6	2.0 (1.4–2.8)
15	338		24.0	31.9	19.6	2.4 (1.4–4.3)

CI: confidence interval; OR: odds ratio; SSR: serotype-specific resistance; TMP-SMX: trimethoprim/sulfamethoxazole.

[a] ORs of antibiotic-resistant serotypes were adjusted for year of isolation, age category and site of isolation (invasive versus non-invasive). Isolates from the 'other' part of Switzerland were used as the reference group.

[b] Isolates with unknown age (n = 681) and/or geographical region known (n = 6) were excluded. Only results for serotypes/serogroups with high SSR are shown (i.e. 19A, 19F, 14, 15, 9V, 23F, 6B, 18C and 33).

serotype/serogroup, using the isolates from the other part of Switzerland as the reference group.

For penicillin, erythromycin and TMP-SMX, three of four (14, 19F and 19A), four of four (33, 15, 19A and 14) and two of four serotypes/serogroups (9V, 23F) showed significantly higher rates in the west than in the rest of the country. This may partly explain the higher non-susceptibility rates for all antibiotics found in the west compared with the rest of the country (Table 2).

Discussion

Our study concurrently compared antibiotic resistance rates from the non-invasive and invasive pneumococcal surveillance systems in Switzerland from 2004 to 2014. Overall, serotype/serogroup and antibiotic resistance data was available for more than 13,000 non-invasive or invasive isolates. The main findings of this study were (i) that antibiotic resistance of pneumococci decreased after the vaccination recommendation of PCV7 and PCV13 and (ii) that patient age and geographical origin of samples had a greater influence on the epidemiology of antibiotic resistance of pneumococci than the site of isolation (i.e. invasive or non-invasive).

Compared with other European countries, the antibiotic resistance rates of the isolates were generally lower in our setting [11]. The French pneumococcal surveillance network analysed the antibiotic susceptibility of 6,683 *S. pneumoniae* isolated from children with acute otitis media from 2001 to 2011 [12]. Non-susceptibility (intermediate and full resistance) rates in 2011 were 57.3% for penicillin in these French isolates. It is generally difficult to compare absolute penicillin resistance data from different studies from different countries because the guidelines for the interpretation of antibiotic resistance have changed (e.g. to apply CLSI versus European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints) However, we identified lower penicillin non-susceptibility rates of 14% within our non-invasive isolates in our study compared with the French study. One possible explanation for these discrepancies between two neighbouring countries might be outpatient antibiotic use which is very low in Switzerland compared with other European countries such as France [13].

Furthermore, we revealed that antibiotic resistance decreased over time in patients up to age 64 years from 2004 to 2014. This can be attributed to the decrease of non-susceptible serotypes such as 19A, 9V, 6B, 23F and 14 among invasive and non-invasive *S. pneumoniae*, which is most likely due to PCV7 and PCV13 vaccination as seen in other studies [12,14-18]. However, it has to be noted that in patients older than 64 years with invasive *S. pneumoniae*, with the exception of TMP-SMX and ceftriaxone, resistance rates remained unchanged from 2004 to 2014, although a serotype redistribution took place also in this age group [4,19]. In the future, serotypes 6C and serogroups 15 and 35 should be followed up carefully as they are the most prominent non-PCV13 serotypes associated with antibiotic resistance. Serotype 6C and serogroup 15 have been implicated in other recent studies as emerging, resistant serotypes [20,21].

Whether invasive and non-invasive pneumococcal isolates inherently differ in resistance rates is still unclear [22,23]. In our study, differences in the ranking of ORs for penicillin, TMP-SMX and erythromycin between

non-invasive and invasive *S. pneumoniae* were minimal, after correcting for age, time and geographical region. Indeed, the rankings of the ORs of the more resistant serotypes for each of the three antibiotics were identical for invasive and non-invasive *S. pneumoniae*. However, the specific setting in which non-invasive isolates were received may be crucial. While we used isolates collected in primary care in situations that do not normally warrant a microbiological diagnosis (uncomplicated otitis media and community-acquired pneumonia), many other studies used data from non-invasive pneumococci generated in routine microbiological work-up [7,24,25]. It is well known that using routine microbiological data may select for more complicated and more often pre-treated cases, which in turn increases the chance of finding antibiotic-resistant isolates [26,27]. We therefore think that the design of our network may reflect a more general situation of antibiotic resistance for these isolates.

Besides temporal trends, we also analysed geographical differences in resistance rates. We found that resistance rates were higher in the west than in the rest of Switzerland. We saw only minor dissimilarities in overall serotype distribution, which cannot explain these geographical differences (data not shown). Therefore, an explanation for these discrepancies could be varying antibiotic consumption. Indeed, higher overall antibiotic consumption has been observed in western Switzerland in in- [28] and outpatients [13,29]. More specifically, a higher antibiotic consumption in the west was found for beta-lactams and macrolides but not for TMP-SMX. This is in line with the higher serotype/serogroup-specific resistance against penicillin and erythromycin but not against TMP-SMX that we observed in western Switzerland. A clear correlation between antibiotic resistance and outpatient use of penicillin has been described previously [11].

This study has some major strengths. It includes a large data volume, allowing for demographic and temporal stratification and the parallel analysis of data from invasive and non-invasive isolates with a great statistical power. Furthermore, it includes congruent analysis of invasive and non-invasive samples in the same laboratory with the same methodology and constantly over a long observation time of 11 years, spanning the introduction of PCV7 and PCV13 vaccines. The non-invasive samples were true outpatient samples and were collected by ca 200 practitioners, representative of Swiss primary care physicians.

There are some limitations to this study. Due to the characteristics of the collection of *S. pneumoniae* isolates, patients with invasive *S. pneumoniae* differed significantly in age and regional origin from those with colonising *S. pneumoniae*. However, the large number of included isolates still allowed for subgroup analysis and adjusting for potential confounding factors in logistic regression models. In addition, colonising *S. pneumoniae* were collected from patients with pneumococcal disease (acute otitis media or pneumonia) but not from those with other diseases such as acute exacerbation of chronic obstructive pulmonary disease (AECOPD) [5,16] or from healthy people. Not including patients with AECOPD may reduce observed rates of antibiotic non-susceptibility as these patients are more likely to have received antibiotics (as discussed above) [26].

Conclusion

In summary, this is a detailed and comprehensive study analysing antibiotic resistance rates and serotype distribution with high statistical power in invasive and non-invasive *S. pneumoniae* in Switzerland. We revealed that antibiotic non-susceptibility was highest in children under five years of age. Furthermore, we showed that variations in the regional antibiotic consumption and the introduction of vaccines may have influenced antibiotic non-susceptibility rates. The introduction of PCV7 and PCV13 markedly reduced resistance rates in pneumococci in Switzerland, mainly by decreasing the prevalence of the most resistant serotypes/serogroups. Site of isolation (invasive vs non-invasive) by itself had a much more limited influence on the epidemiology. At the end of the study period in 2014, distinct antibiotic resistance rates were on an all-time low for various age groups, but certain non-PCV13 resistant serotypes have to be carefully monitored in the future. The two described national surveillance systems are both important to guide future antibiotic stewardship and vaccination policies in Switzerland.

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Conflict of interest

An educational grant from Pfizer AG for partial support of this project was received. However, the sponsor had no role in the data analysis and content of the manuscript.

Authors' contributions

All authors conceived and designed the study; CH, AK, MH analysed the data and wrote the manuscript. CH, AK, AA, MH contributed to the discussion and reviewed the manuscript. CH, AK, AA, MH saw, commented upon and approved the final version of the paper.

References

1. Aebi C, Duppenthaler A. [Antimicrobial resistance--consequences for ambulatory treatment of infections in children]. Ther Umsch. 2002;59(1):46-50. German. DOI: 10.1024/0040-5930.59.1.46 PMID: 11851047
2. Feikin DR, Schuchat A, Kolczak M, Barrett NL, Harrison LH, Lefkowitz L, et al. Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995-1997. Am J Public Health. 2000;90(2):223-9. DOI: 10.2105/AJPH.90.2.223 PMID: 10667183
3. Schwaber MJ, De-Medina T, Carmeli Y. Epidemiological interpretation of antibiotic resistance studies - what are we missing?Nat Rev Microbiol. 2004;2(12):979-83. DOI: 10.1038/nrmicro1047 PMID: 15550943
4. Meichtry J, Born R, Küffer M, Zwahlen M, Albrich WC, Brugger SD, et al. Serotype epidemiology of invasive pneumococcal disease in Swiss adults: a nationwide population-based study. Vaccine. 2014;32(40):5185-91. DOI: 10.1016/j.vaccine.2014.07.060 PMID: 25077419
5. Song JH, Dagan R, Klugman KP, Fritzell B. The relationship between pneumococcal serotypes and antibiotic resistance.Vaccine. 2012;30(17):2728-37. DOI: 10.1016/j.vaccine.2012.01.091 PMID: 22330126
6. Kronenberg A, Zucs P, Droz S, Mühlemann K. Distribution and invasiveness of Streptococcus pneumoniae serotypes in Switzerland, a country with low antibiotic selection pressure, from 2001 to 2004.J Clin Microbiol. 2006;44(6):2032-8. DOI: 10.1128/JCM.00275-06 PMID: 16757594
7. Hoban DJ, Doern GV, Fluit AC, Roussel-Delvallez M, Jones RN. Worldwide prevalence of antimicrobial resistance in Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis in the SENTRY Antimicrobial Surveillance Program, 1997-1999.Clin Infect Dis. 2001;32(Suppl 2):S81-93.
8. Mühlemann K, Matter HC, Täuber MG, Bodmer T, Sentinel Working Group. Nationwide surveillance of nasopharyngeal Streptococcus pneumoniae isolates from children with respiratory infection, Switzerland, 1998-1999.J Infect Dis. 2003;187(4):589-96. DOI: 10.1086/367994 PMID: 12599075
9. Bundesamt für Gesundheit (BAG). Repräsentativität des Sentinella-Ärztekollektivs. [Representativeness of the collective of sentinel physicians]. BAG Bulletin. 2001;37(01):680. German. Available from: http://www.bag.admin.ch/dokumentation/publikationen/01435/01801/index.html?lang=de&download=NHzLpZig7t,lnp6IoNTU042l2Z6ln1acy4Zn4Z2qZpnO2Yuq2Z6gpJCEdX56fWym162dpYbUzd,Gpd6emK2Oz9aGodetmqaN19XI2IdvoaCUZ,s-
10. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, twenty-second informational supplement. Document M100-S22. Wayne: CLSI; 2012
11. Goossens H, Ferech M, Vander Stichele R, Elseviers M, ESAC Project Group. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study.Lancet. 2005;365(9459):579-87. DOI: 10.1016/S0140-6736(05)70799-6 PMID: 15708101
12. Kempf M, Varon E, Lepoutre A, Gravet A, Baraduc R, Brun M, et al. Decline in antibiotic resistance and changes in the serotype distribution of Streptococcus pneumoniae isolates from children with acute otitis media; a 2001-2011 survey by the French Pneumococcal Network. Clin Microbiol Infect. 2015;21(1):35-42. DOI: 10.1016/j.cmi.2014.08.009 PMID: 25636925
13. Filippini M, Masiero G, Moschetti K. Socioeconomic determinants of regional differences in outpatient antibiotic consumption: evidence from Switzerland.Health Policy. 2006;78(1):77-92. DOI: 10.1016/j.healthpol.2005.09.009 PMID: 16290129
14. Imöhl M, Reinert RR, Mutscher C, van der Linden M. Macrolide susceptibility and serotype specific macrolide resistance of invasive isolates of Streptococcus pneumoniae in Germany from 1992 to 2008.BMC Microbiol. 2010;10(1):299. DOI: 10.1186/1471-2180-10-299 PMID: 21108778
15. Kyaw MH, Lynfield R, Schaffner W, Craig AS, Hadler J, Reingold A, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant Streptococcus pneumoniae. N Engl J Med. 2006;354(14):1455-63. DOI: 10.1056/NEJMoa051642 PMID: 16598044
16. Hackel M, Lascols C, Bouchillon S, Hilton B, Morgenstern D, Purdy J. Serotype prevalence and antibiotic resistance in Streptococcus pneumoniae clinical isolates among global populations.Vaccine. 2013;31(42):4881-7. DOI: 10.1016/j.vaccine.2013.07.054 PMID: 23928466
17. Imöhl M, Reinert RR, van der Linden M. Serotype-specific penicillin resistance of Streptococcus pneumoniae in Germany from 1992 to 2008.Int J Med Microbiol. 2010;300(5):324-30. DOI: 10.1016/j.ijmm.2009.11.004 PMID: 20071233
18. Dagan R, Juergens C, Trammel J, Patterson S, Greenberg D, Givon-Lavi N, et al. Efficacy of 13-valent pneumococcal conjugate vaccine (PCV13) versus that of 7-valent PCV (PCV7) against nasopharyngeal colonization of antibiotic-nonsusceptible Streptococcus pneumoniae. J Infect Dis. 2015;211(7):1144-53. DOI: 10.1093/infdis/jiu576 PMID: 25355940
19. Bundesamt für Gesundheit (BAG). Pneumokokken-Erkrankungen. [Pneumococcal diseases]. Bern: BAG; 2013. German. Available from: http://www.bag.admin.ch/themen/medizin/00682/00684/01097/index.html?lang=it
20. Chiba N, Morozumi M, Shouji M, Wajima T, Iwata S, Ubukata K, et al. Changes in capsule and drug resistance of Pneumococci after introduction of PCV7, Japan, 2010-2013. Emerg Infect Dis. 2014;20(7):1132-9. DOI: 10.3201/eid2007.131485 PMID: 24960150
21. Regev-Yochay G, Paran Y, Bishara J, Oren I, Chowers M, Tziba Y, et al. Early impact of PCV7/PCV13 sequential introduction to the national pediatric immunization plan, on adult invasive pneumococcal disease: A nationwide surveillance study. Vaccine. 2015;33(9):1135-42. DOI: 10.1016/j.vaccine.2015.01.030 PMID: 25613717
22. Lambertsen LM, Harboe ZB, Konradsen HB, Christensen JJ, Hammerum AM. Non-invasive erythromycin-resistant pneumococcal isolates are more often non-susceptible to more antimicrobial agents than invasive isolates. Int J Antimicrob Agents. 2010;35(1):72-5. DOI: 10.1016/j.ijantimicag.2009.09.006 PMID: 19892529
23. Saha SK, Baqui AH, Darmstadt GL, Ruhulamin M, Hanif M, El Arifeen S, et al. Comparison of antibiotic resistance and serotype composition of carriage and invasive pneumococci among Bangladeshi children: implications for treatment policy and vaccine formulation. J Clin Microbiol. 2003;41(12):5582-7. DOI: 10.1128/JCM.41.12.5582-5587.2003 PMID: 14662944
24. Jacobs MR, Felmingham D, Appelbaum PC, Grüneberg RN, Alexander Project Group. The Alexander Project 1998-2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents.J Antimicrob Chemother. 2003;52(2):229-46. DOI: 10.1093/jac/dkg321 PMID: 12865398
25. Beekmann SE, Heilmann KP, Richter SS, García-de-Lomas J, Doern GV, GRASP Study Group. Antimicrobial resistance in Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and group A beta-haemolytic streptococci in 2002-2003. Results of the multinational GRASP Surveillance Program.Int J Antimicrob Agents. 2005;25(2):148-56. DOI: 10.1016/j.ijantimicag.2004.09.016 PMID: 15664485
26. Pérez-Trallero E, Marimón JM, Larruskain J, Alonso M, Ercibengoa M. Antimicrobial susceptibilities and serotypes of Streptococcus pneumoniae isolates from elderly patients with pneumonia and acute exacerbation of chronic obstructive pulmonary disease.Antimicrob Agents Chemother. 2011;55(6):2729-34. DOI: 10.1128/AAC.01546-10 PMID: 21402843
27. Kronenberg A, Koenig S, Droz S, Mühlemann K. Active surveillance of antibiotic resistance prevalence in urinary tract and skin infections in the outpatient setting.Clin Microbiol Infect. 2011;17(12):1845-51. DOI: 10.1111/j.1469-0691.2011.03519.x PMID: 21880098
28. Plüss-Suard C, Pannatier A, Kronenberg A, Mühlemann K, Zanetti G. Hospital antibiotic consumption in Switzerland: comparison of a multicultural country with Europe.J Hosp Infect. 2011;79(2):166-71. DOI: 10.1016/j.jhin.2011.05.028 PMID: 21820207
29. Achermann R, Suter K, Kronenberg A, Gyger P, Mühlemann K, Zimmerli W, et al. Antibiotic use in adult outpatients in Switzerland in relation to regions, seasonality and point of care tests. Clin Microbiol Infect. 2011;17(6):855-61. DOI: 10.1111/j.1469-0691.2010.03348.x PMID: 20731682

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Research article

Changing characteristics of livestock-associated meticillin-resistant *Staphylococcus aureus* isolated from humans – emergence of a subclade transmitted without livestock exposure, the Netherlands, 2003 to 2014

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Since 2007, livestock-associated meticillin-resistant *Staphylococcus aureus* (LA-MRSA) has become the predominant MRSA clade isolated from humans in the Netherlands. To assess possible temporal changes, we molecularly characterised over 9,000 LA-MRSA isolates submitted from 2003 to 2014 to the Dutch MRSA surveillance. After an initial rapid increase with a peak in 2009 (n=1,368), the total number of submitted LA-MRSA isolates has been slowly decreasing to 968 in 2014 and over 80% of LA-MRSA belonged to one of three predominant MLVA/spa-types. Next generation sequencing (n=118) showed that MT569/t034 isolates were genetically more diverse than MT398/t011 and MT572/t108. Concurrent with the decrease in LA-MRSA, fewer people reported having contact with livestock and this was most prominent for people carrying MT569/t034 LA-MRSA. The proportion of LA-MRSA isolated from infection-related materials increased from 6% in 2009, to 13% in 2014 and most of these isolates originated from patients older than 50 years of age. Remarkably, 83% of these patients reported not having contact with livestock. The results reveal an ongoing change in the genotypic and epidemiological characteristics of Dutch LA-MRSA isolated from humans with the emergence of a LA-MRSA subclade independent of livestock exposure, suggesting LA-MRSA starts to resemble non-LA-MRSA in terms of transmissibility and pathogenicity.

Introduction

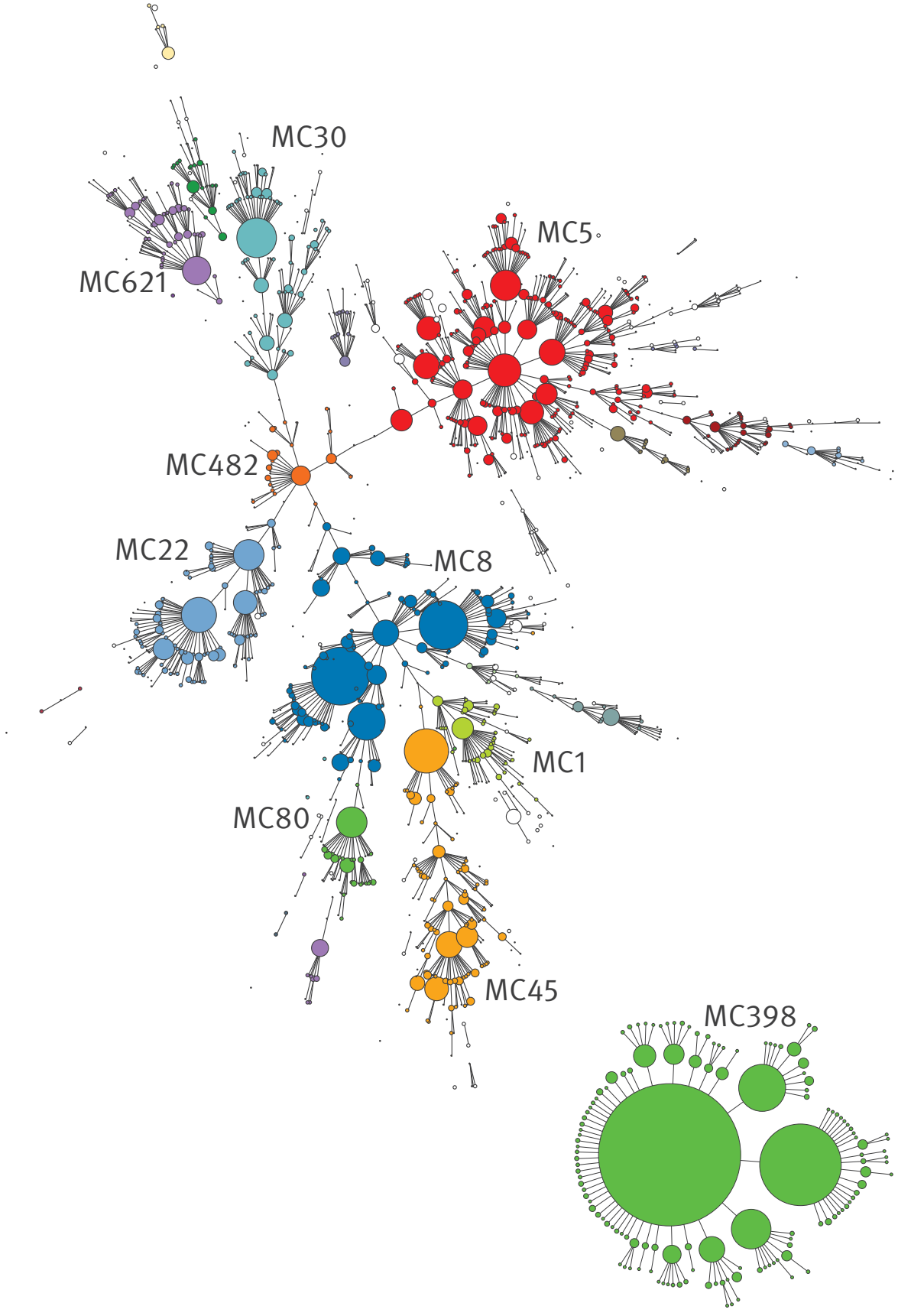
Meticillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital-acquired and community-acquired infections [1]. In 2003, a clonal lineage of MRSA cultured from pig farmers and designated as multilocus sequence typing clonal complex 398 (CC398), emerged in the Netherlands and France [2,3]. A large number of countries reported CC398 cultured from animals, revealing a worldwide prevalence [4,5]. CC398 has been found in pigs and other livestock, such as calves, and poultry [6,7], and therefore designated as livestock-associated MRSA (LA-MRSA). LA-MRSA is prevalent in many European countries and LA-MRSA CC398 isolated from humans has become the predominant MRSA clade among isolates submitted for typing in the Dutch MRSA surveillance programme since 2007 [5,8].

Despite its widespread occurrence, the number of reported infections with LA-MRSA among humans remains low. In addition, nosocomial transmission of LA-MRSA in Dutch hospitals was reported to be 72% less likely to occur compared with non-LA-MRSA, although outbreaks of LA-MRSA have been described [9,10]. The reason for this limited transmissibility remains unclear, but several studies suggest that the human innate immunomodulatory genes on bacteriophage φ3 are important genetic markers for the adaptation of LA-MRSA towards humans and human-to-human transmission of CC398 [11-13].

LA-MRSA represents a homogenous clade with limited differentiation using typing techniques such as multiple-locus variable number of tandem repeat analysis (MLVA), and *staphylococcal* protein A (*spa*-) typing [14]. In contrast, whole genome mapping, and next generation sequencing (NGS), revealed more genotypic diversity and suggested a distinction between livestock- and human-associated CC398 clades, although most studies are dominated by isolates obtained from animals and the human-associated isolates were mostly comprised of meticillin-sensitive *S. aureus* [12,14-16].

Figure 1

Minimum spanning tree of *Staphylococcus aureus* isolates typed by MLVA, the Netherlands, 2008–14 (n= 22,945)

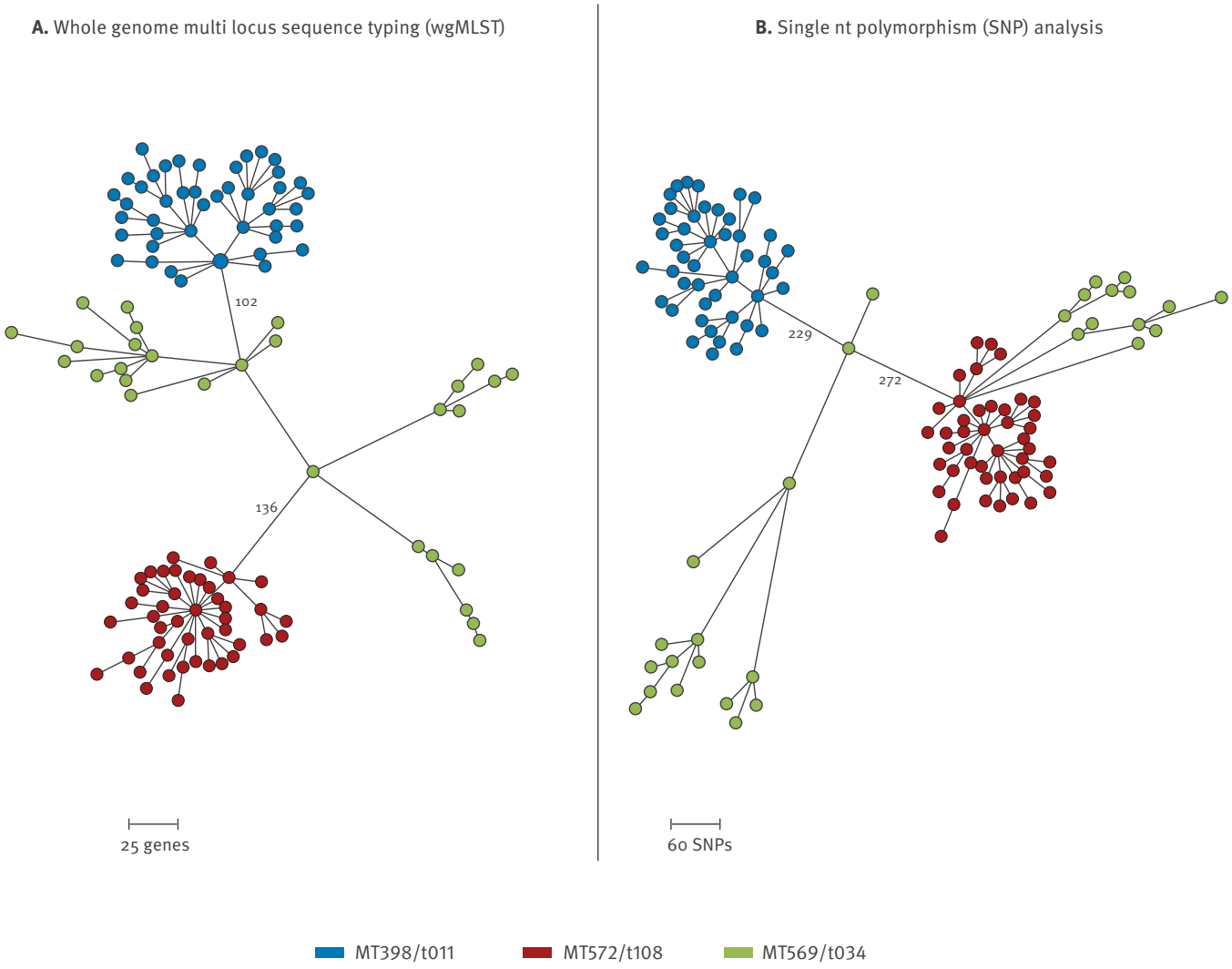


LA-MRSA: livestock-associated meticillin-resistant Staphylococcus aureus; MC: MLVA complex; MLVA: multiple-locus variable number of tandem repeat analysis.

Clustering of MLVA profiles was done using a categorical coefficient and the MLVA types are displayed as circles. The size of each circle indicates the number of isolates with this particular type. Colours denote MLVA types that belong to the same MLVA complex, which are also indicated in characters e.g. MC398. Due to the large number of MC398 isolates, representing LA-MRSA, this complex is displayed separately.

Figure 2

Minimum spanning trees based on next generation sequencing of LA-MRSA isolates, the Netherlands, 2003–12 (n=118)



LA-MRSA: livestock-associated meticillin-resistant Staphylococcus aureus; MLVA: multiple-locus variable number of tandem repeat analysis; MT: MLVA type; SNP: single nucleotide polymorphism; wgMLST: whole genome multilocus sequence typing.

Panel A displays the minimum spanning tree based on 1,831 genes of the S. aureus wgMLST scheme, while panel B shows the tree based on 7,944 SNPs. Colours represent the top 3 representatives of LA-MRSA, MT398/t011 (n=45), MT572/t108 (n=44) and MT569/t034 (n=29). The lengths of the lines between isolates represent either the number of different genes (wgMLST) or the number of SNPs.

In this study, molecular characterisation, including NGS, and epidemiological data of more than 9,000 LA-MRSA isolates submitted to the national MRSA surveillance from 2003 to 2014 were used to assess the characteristics of the most predominant MRSA clade in the Netherlands.

Methods

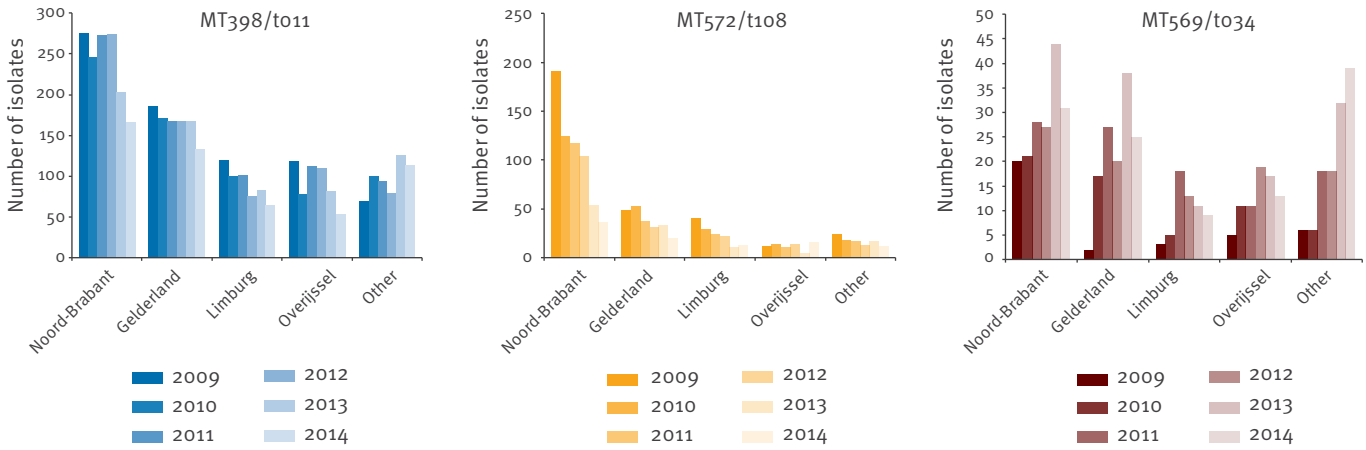
Bacterial isolates

MRSA isolates, obtained from humans admitted to healthcare centers, were submitted for molecular typing to the National Institute of Public Health and the Environment (RIVM) to the Dutch national MRSA surveillance. All *S. aureus* isolates were subjected to *spa*-typing, and MLVA. The MLVA also includes the detection of the genes for *mec*A, *mec*C and the *luk*F gene, indicative for Panton-Valentine leucocidin (PVL) [15,16]. Isolates belonging to MLVA complex 398 (MC398) were classified as LA-MRSA. All isolates not belonging to MC398 were designated as non-LA-MRSA. The discriminatory power of MLVA was assessed using Simpson's index of diversity, while the determination of the confidence intervals (CI) of the Simpson's indices was calculated as described by Grundmann et al. [17,18]. Only the first isolate per person per year was included. Medical microbiologists or infection control practitioners filled out questionnaires regarding epidemiological risk factors for MRSA colonisation or infection, including contact with livestock.

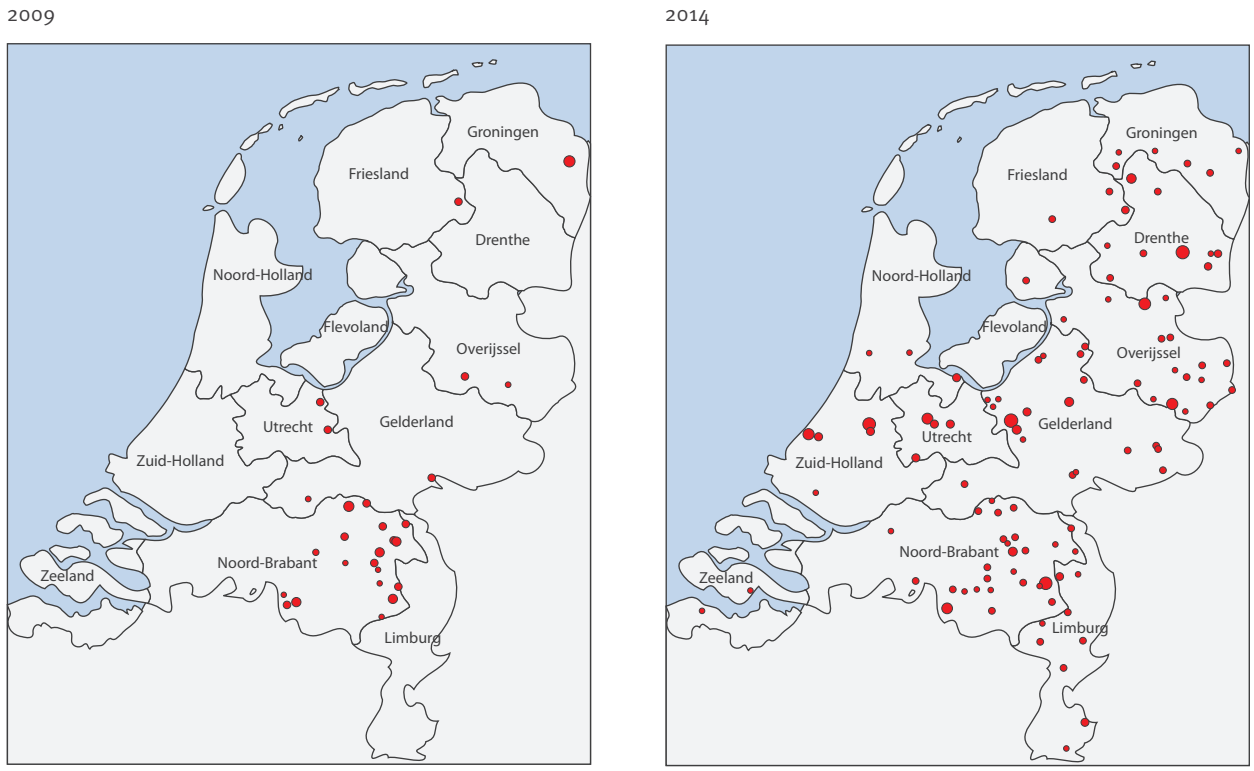
Figure 3

Geographic origin of the top three LA-MRSA genotypes by provinces, the Netherlands, 2009 and 2014

A. LA-MRSA genotypes



B. The geographic demographic origin of LA-MRSA MT569/to34 isolates

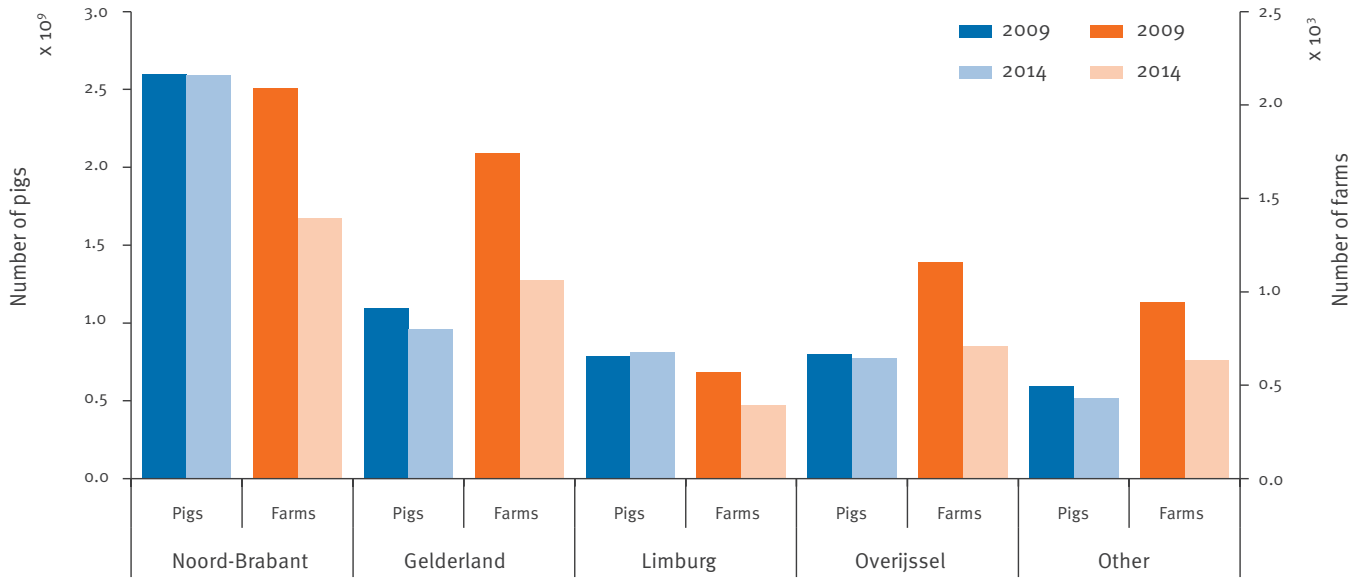


LA-MRSA: livestock-associated meticillin-resistant Staphylococcus aureus; MLVA: multiple-locus variable number of tandem repeat analysis; MT: MLVA type.

The number of submitted isolates of the top-three LA-MRSA representatives in the four provinces where LA-MRSA was predominant vs the rest of the Netherlands is shown in panel A. The geographic origin of LA-MRSA MT569/to34 isolates in the Netherlands is depicted in panel B.

Figure 4

Number of pig farms and number of pigs by provinces, the Netherlands, 2009 and 2014



Data source: statline.cbs.nl/statweb.

Classification of materials

The MRSA isolates were sampled from various materials and sites. Materials were subdivided in four different classes. Swabs from nose and/or throat, and/or perineum were regarded as material indicative for 'carriage'. Blood, cerebrospinal fluid (CSF), pus, sputum, urine, and wounds were considered as 'infection-related' materials. Other human materials were grouped as 'other' and if submitted without information regarding material, they were grouped as 'unknown'.

Next generation sequencing

The first 10 (if available) isolates of the three predominant LA-MRSA MLVA/*spa*-types from 2003 to 2005 and the first five isolates from 2006 to 2012 were used for analysis by NGS. NGS on these 118 isolates was performed as part of the 100k genome project by Davis University using the Hiseq 2000 [19]. Data were used for whole genome multilocus sequence typing (wgMLST), and single nucleotide polymorphism (SNP-) analysis. wgMLST was performed by SeqSphere software version 2.3.0 (Ridom GmbH, Münster, Germany) using the available wgMLST *S. aureus* scheme. For SNP analysis, the core genome, of a complete, circular and annotated reference chromosome of a Dutch LA-MRSA isolate was used. SNPs were identified using the CLCbio Genomics Server/Workbench, version 7.5 (CLCbio, Aarhus, Denmark) and SNP data were imported into Bionumerics version 7.5 for analysis (Applied Maths, Sint-Martens-Latem, Belgium).

φ3-specific PCR

We designed the φ3-specific primers based on the φ3 sequence present in reference strain NCTC8325 (CP000253): fluorescently labelled forward primer Sa3-Int-PET-f (TGATTTGTACGGGTTGTC), and the reverse primer Sa3-Int-r (TACTTATGACGTCCATAATGTG). The φ3 primers (10 pmol/µl) were added in our MLVA mix2, allowing detection of φ3 as a 160 bp peak. All LA-MRSA and non-LA-MRSA isolates obtained from August 2012 to October 2013 were tested for the presence of φ3.

Results

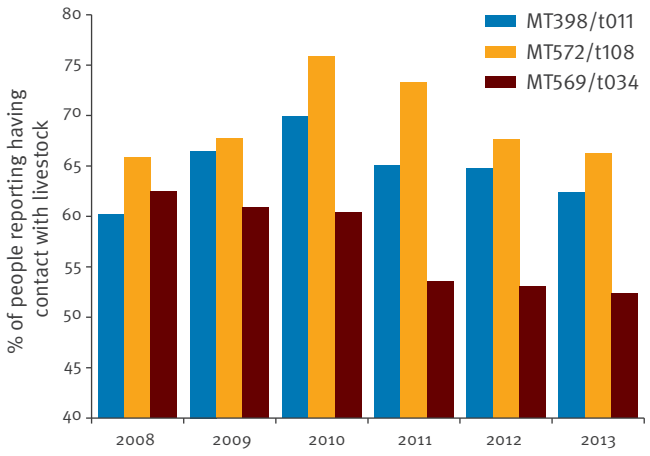
Genotypic diversity of LA-MRSA vs non-LA-MRSA

During the study period, we identified 17,079 isolates as non-LA-MRSA and 10,318 as LA-MRSA. Inclusion of only the first isolate per person per year resulted in 9,246 LA-MRSA isolates, and 13,699 non-LA-MRSA isolates to be analysed in this study for the period from 2003 to 2014. Questionnaires were available for 5,958 persons from whom LA-MRSA was isolated during the period from 2006 to 2013.

Genotypic diversity among non-LA-MRSA isolates was higher than LA-MRSA (Figure 1). For instance, MC5 (n = 3,202), the most frequently found non-LA-MRSA MLVA complex, comprised 244 MLVA-types (MTs). In contrast, MLVA yielded only 144 MTs among the 9,246 LA-MRSA (MC398) isolates, resulting in a diversity index (DI) of 0.64 (95% CI: 0.63–0.65). The predominant MT was MT398 (n = 5,111, 55%), followed by MT572 (n = 1,872, 20%), and MT569 (n = 603, 7%).

Figure 5

Changes in the proportion of people carrying LA-MRSA who report having contact with livestock, the Netherlands, 2008–13



LA-MRSA: livestock-associated meticillin-resistant Staphylococcus aureus; MLVA: multiple-locus variable number of tandem repeat analysis.

The proportion is expressed as the percentage of people who reported having livestock contact per MLVA/spa-type for the top three LA-MRSA types.

Spa-typing was slightly less discriminatory yielding 120 *spa*-types (DI = 0.60, 95% CI: 0.59–0.61). *Spa*-types t011 (n = 5,422, 59%), t108 (n = 1,860, 20%), and t034 (n = 723, 8%) were predominant.

Based on MLVA and *spa*-typing combined, the three predominant LA-MRSA types were MT398/t011 (n = 5,043, 55%), MT572/t108 (n = 1,742, 19%), and MT569/t034 (n = 594, 6%). These top three types accounted for 80% of all LA-MRSA isolates. No other combination of MT and *spa*-type accounted for more than 2% of the LA-MRSA isolates.

Panton-Valentine leukocidin and φ3 in Dutch LA-MRSA isolates

The presence of the *lukF* gene, indicative for the production of the Panton-Valentine leukocidin (PVL), was determined in all 9,246 LA-MRSA isolates, but found in only 23 (0.2%) with a great variety in MLVA/*spa*-types. Ten of the 23 PVL positives originated from persons younger than 10 years, four of whom were adoption children from China. The proportion of non-LA-MRSA isolates carrying the *lukF* gene was 26% (n=3,585).

Between August 2012 and October 2013 1,538 LA-MRSA and 3,405 non-LA-MRSA isolates were tested for the presence of φ3. The prevalence of φ3 among LA-MRSA isolates was 2% (34/1,538). There was a difference in φ3 prevalence among the top three MLVA/*spa*-types; 7% (11/166) in MT569/t034, 2% (13/838) in MT398/t011, and 0.6% (1/180) in MT572/t108 isolates. In contrast, prevalence in non-LA-MRSA was much higher with 80% (2,714/3,405) of all tested isolates carrying φ3.

Whole genome multilocus sequence typing and single nucleotide polymorphism analysis

Both wgMLST and SNP analysis of 118 isolates of the three predominant LA-MRSA types showed that they clustered in three different groups (Figure 2). Of the 1,864 genes of the *S. aureus* wgMLST scheme, 1,831 were present in all 118 isolates and used for comparison and tree construction. LA-MRSA isolates belonging to MT398/t011 (n = 45) clustered closely together as did MT572/t108 (n=44) isolates. The average distance between MT398/t011 isolates was allelic variation in 25 genes with a maximum of 60 genes and for MT572/t108, the average distance was 24 genes with a maximum of 52 genes. The closest related isolates of the two groups differed in 145 genes. Compared with MT398/t011 and MT572/t108, the genetic diversity among the MT569/t034 (n = 29) isolates was higher. The MT569/t034 isolates differed on average 34 genes with a maximum of 148 genes. The distances between MT569/t034 and MT398/t011, and MT572/t108 were 102 and 136 genes, respectively.

We identified 7,944 SNP positions in the core genome and used these for comparison. The minimum spanning tree was comparable to the wgMLST tree with groups of closely related MT398/t011, and MT572/t108 isolates and a genetic diverse MT569/t034. The average number of SNPs that differed among members of the MT398/t011 group was 43 with a maximum of 117 SNPs, while the MT572/t108 isolates differed on average in 39 SNPs with a maximum of 80 SNPs between the most distant members. The distance between the closest related isolates of MT398/t011, and MT572/t108 was 274 SNPs.

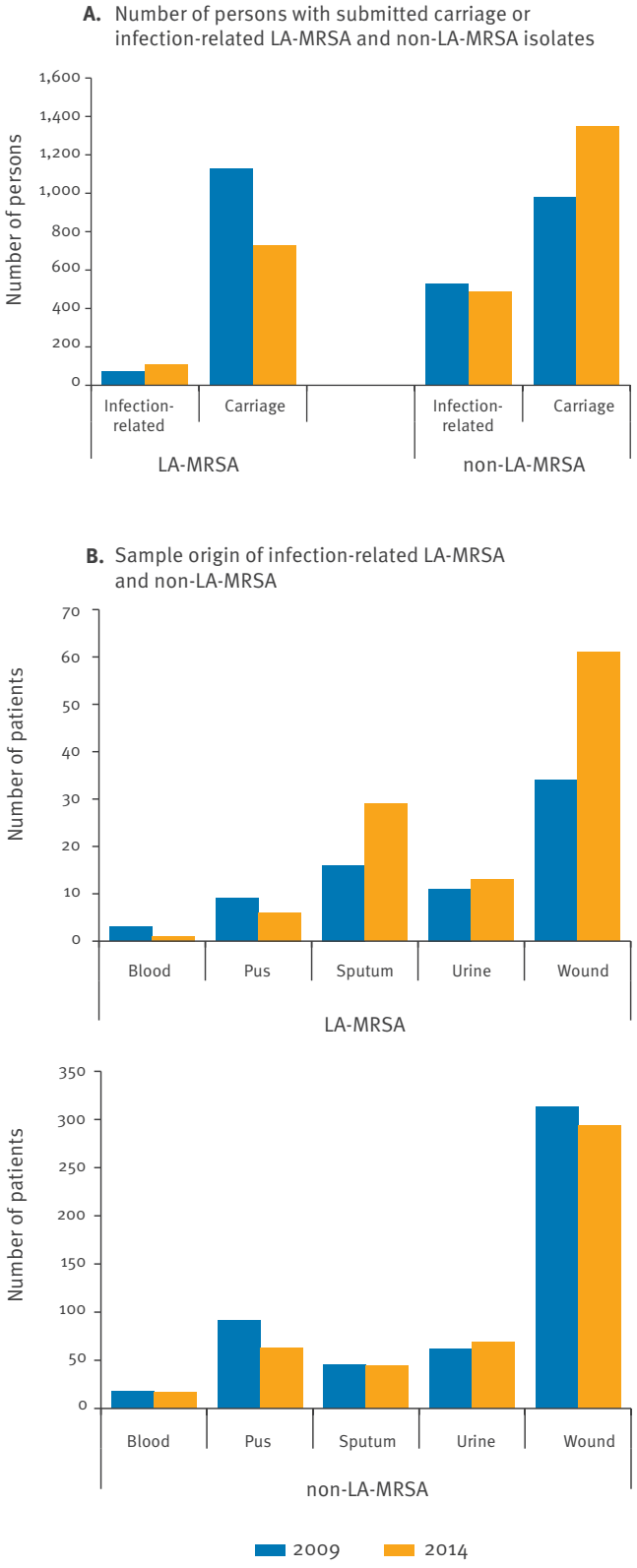
Decrease in the number of submitted LA-MRSA isolates

After its emergence in 2003, the number of LA-MRSA isolates submitted for typing rapidly increased from 20 in 2003 to 1,019 in 2008. At its peak, in 2009, 1,368 of the 3,163 (43%) MRSA isolates (first isolate per person per year) sent to the RIVM were LA-MRSA, but since then the numbers dropped. In 2014, the total number of submitted MRSA isolates was 3,228, of which 968 (30%) were LA-MRSA. The decrease could be largely attributed to the drop in submitted MT398/t011, and MT572/t108 isolates. In contrast, the number of isolates with MT569/t034 has been increasing since 2008. In 2014, 12% (n=117) of the 968 LA-MRSA isolates were of MT569/t034, surpassing MT572/t108 as the second most frequently isolated Dutch LA-MRSA type.

A geographical comparison between 2009 and 2014 showed a steady decrease of MT398/t011 in the four provinces, Noord-Brabant, Gelderland, Limburg, and Overijssel, where LA-MRSA is predominant and a slight increase in the other Dutch provinces (Figure 3A). This was most prominent in the province of Noord-Brabant

Figure 6

Sample origin of submitted LA-MRSA and non-LA-MRSA isolates, the Netherlands, 2009 and 2014 (n=5,391)



LA-MRSA: livestock-associated meticillin-resistant *Staphylococcus aureus*.

where a decrease of 40% occurred over time. A similar trend was seen for MT572/t108 LA-MRSA, a type predominantly found in Noord-Brabant resulting in a 69% decrease between 2009 and 2014. In contrast, there was a marked increase until 2013 in the number of submitted MT569/t034 isolates that was not restricted to a particular province. In 2014, the number of isolates slightly decreased in the four provinces, but this did not to occur in the rest of the Netherlands. In 2009, 56% of the MT569/t034 isolates originated from Noord-Brabant, but this dropped to 26% in 2014 (Figure 3B).

Concurrent with the drop in the number of submitted LA-MRSA, a decrease in the number of pig farms in the Netherlands occurred (Figure 4). This decline was most prominent in the provinces Noord-Brabant and Gelderland. In addition, the number of people working in the Dutch agricultural sector has declined by 35% between 2000 and 2014 [20]. However, the number of pigs remained stable over time, showing a scale up in the Dutch pig production.

LA-MRSA related to contact with livestock

In 2008, 60–66% of persons carrying any of the three predominant LA-MRSA types reported contact with livestock. When stratified by the top three MLVA/*spa*-types an initial increase of contact with livestock from 2008 to 2010 was reported for people carrying isolates with MT398/t011, and MT572/t108 (Figure 5). After 2010, the proportion of humans reporting contact with livestock decreased again for both types reaching 62% (244/391) and 66% (49/74) for MT398/t011, and MT572/t108, respectively. In contrast, a considerable decrease in reported livestock contact occurred in people with MT569/t034 LA-MRSA where the percentage dropped from 63% (5/8) in 2008 to 52% (44/84) in 2013.

Sample origin of LA-MRSA isolates

Most of the isolates submitted for typing originated from carriage-related materials. In 2009, 6% (76/1,205) of the LA-MRSA isolates were cultured from infection-related materials and despite a drop in the number of LA-MRSA, this proportion increased to 13% (111/841) in 2014 (Figure 6A). In contrast, the number of non-LA-MRSA isolates increased during the same period, yet the proportion of isolates from infection-related materials decreased from 35% (529/1,510) in 2009 to 27% (487/1,835) in 2014.

Of the infection-related LA-MRSA isolates, most samples originated from wounds and sputum (Figure 6B). In 2009, 3% (37/1,205) of the LA-MRSA isolates were cultured from wounds and this increased to 7% (61/841) in 2014. For LA-MRSA isolates originating from sputum, an increase from 1% (16/1,205) in 2009 to 4% (29/841) in 2014 was seen. In contrast, the proportion of wound and sputum samples in the non-LA-MRSA slightly decreased during the same period. The distribution of MLVA/*spa*-types of isolates from infection-related materials did not differ from LA-MRSA isolates obtained from carriage-related materials.

Age-distribution among people carrying LA-MRSA

The median age of people carrying LA-MRSA and those carrying non-LA-MRSA was similar at 48 (range 0 to 105 years), and 49 years (range 2 to 103 years), respectively. However, stratification into age groups revealed a large difference between LA-MRSA carriers and non-LA-MRSA carriers (Figure 7A). In people carrying LA-MRSA, age categories followed a Gaussian distribution with a peak at 41–50 years. The proportion of infection-related isolates increased with increasing age, from 5% in the 0–9 years age group to 44% in the 80–89 years age group. In contrast, there was an almost even age distribution in persons carrying non-LA-MRSA and the proportion of infection-related isolates increased from 18% to 37% in age groups 0–9 years, and 80–89 years, respectively. Remarkably, there was a dip in the age distribution of persons carrying non-LA-MRSA in the age group 11–20 years.

There was a Gaussian age distribution of people carrying LA-MRSA, who reported having contact with livestock, and the vast majority was carriage (Figure 6B). The age groups of people carrying LA-MRSA, who reported not having contact with livestock, were also distributed in a Gaussian fashion, but with lower amplitude. The proportion of infection-related isolates was much higher than in the group of people reporting contact with livestock, increasing from 10% in age group 0–9 years to 59% in the age group 80–89 years. In contrast, in people reporting livestock contact, proportions were only 2%, and 21% in these age groups.

Discussion

In this study, we used a collection of more than 9,000 LA-MRSA isolates originating from humans obtained over the years 2003 to 2014. We showed an increase in the number of MT569/t034 LA-MRSA isolates, despite a decrease in the total number of LA-MRSA isolates in the Netherlands in recent years. NGS demonstrated that MT398/t011 isolates and MT572/t108 isolates partitioned in two genetically homogeneous groups, while MT569/t034 isolates did not partition in a single group and were genetically more diverse. Since 2010, humans carrying LA-MRSA less frequently reported having contact with livestock and this was most prominent for persons carrying MT569/t034 LA-MRSA.

The total number of MRSA isolates submitted for typing to the Dutch MRSA surveillance has been increasing since the start of the surveillance programme in 1989.

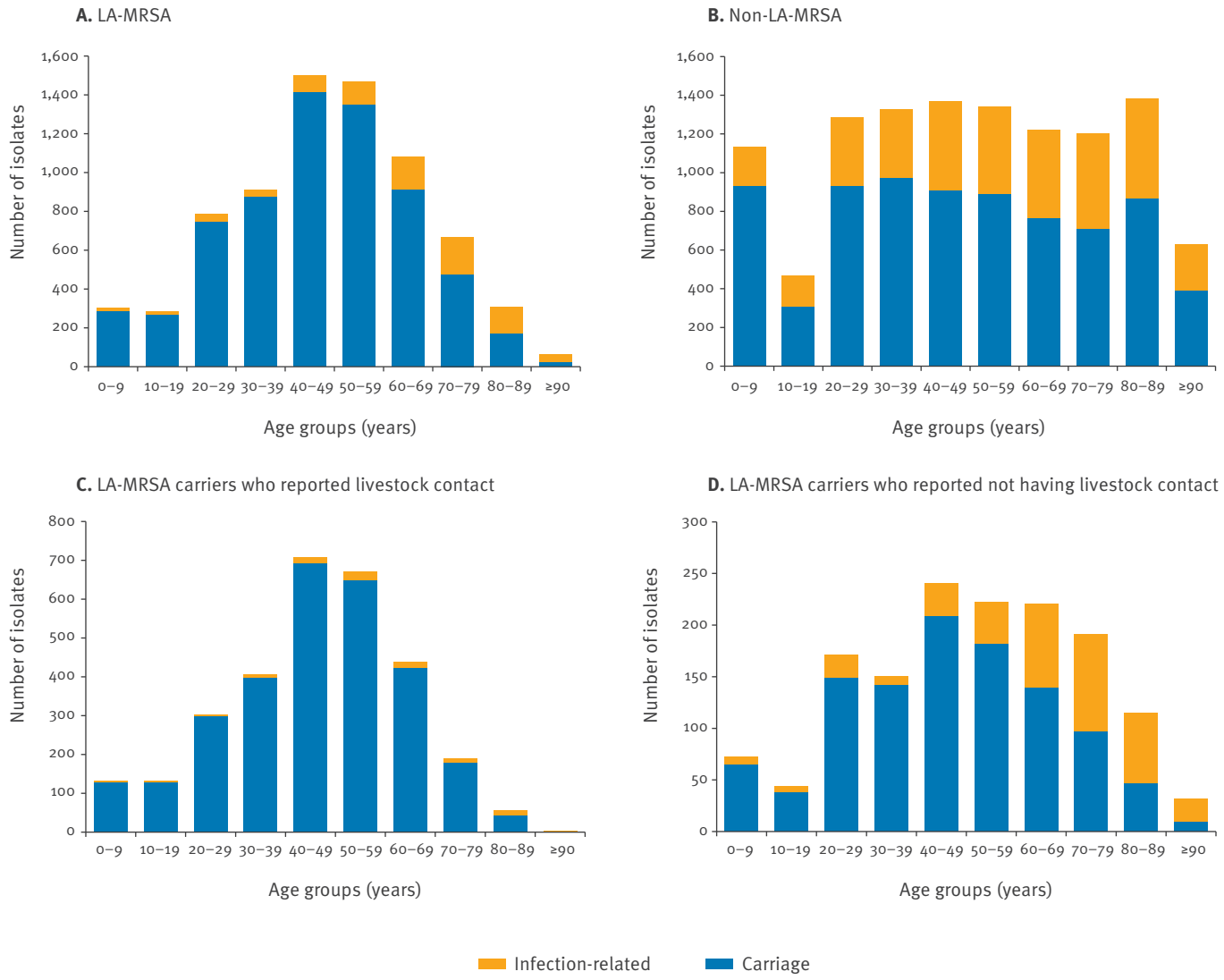
Since the first finding in 2003, the number of submitted LA-MRSA isolates increased rapidly until 2009 when the proportion of LA-MRSA was 43%. After that, the number of submitted LA-MRSA isolates dropped from 1,393 in 2009 to 968 in 2014 and as a result, the proportion of LA-MRSA decreased to 30% in 2014. Possible explanations for this decrease could be a reduced number of persons exposed to LA-MRSA, since we observed a concurrent decline in the number of pig farms and people working the agricultural sector, although the number of pigs did not diminish. Also, there may be a reluctance of medical microbiology laboratories to submit LA-MRSA isolates as current typing poorly discriminates LA-MRSA, transmissibility between humans is considered to be low, and the perception may exist that infections with LA-MRSA occur only sporadically. However, we observed an increase in the number of submitted LA-MRSA isolated from infection-related materials, and this resulted in doubling the proportion of infection-related LA-MRSA from 6% in 2009 to 13% in 2014 and the majority of infection-related LA-MRSA originated from wounds and sputum. This shows that LA-MRSA is not only successful in colonising humans, but is also capable of causing infections. It also suggests that medical microbiology laboratories that have already assessed that an isolate is LA-MRSA, prefer to submit LA-MRSA isolated from infection-related materials rather than carriage isolates. This is not the case for non-LA-MRSA, as the number of submitted carriage-related isolates is increasing, while the number of infection-related isolates remains unchanged.

Analyses of the NGS data of the three predominant LA-MRSA types revealed three different groups. There was no overlap between the types, suggesting that these LA-MRSA types are three unique and independently evolving LA-MRSA clades. The genetically most diverse variant was MT569/t034, the type that rapidly increased in the Netherlands in recent years. This finding suggests that this particular LA-MRSA variant is more adapting towards humans leading to spread to regions of the Netherlands where LA-MRSA is not the predominant MRSA variant and where density of livestock farms is relatively low. Furthermore, the observation that there was a strong decrease in the number of people who reported having contact with livestock while carrying MT569/t034 LA-MRSA suggests that the spread of this LA-MRSA variant also occurs through routes other than livestock-human transmission. Recently, two studies from the Netherlands showed that a large proportion of the MRSA without known origin belonged to the LA-MRSA clade, corroborating our suggestion that LA-MRSA is capable of spreading without livestock exposure [21,22]. The φ3 phage, proposed by several studies as one of the markers for the adaptation of LA-MRSA towards humans, was nearly absent in our collection, although φ3 prevalence was highest (7%) among MT569/t034 isolates [11,12,23]. This suggests that φ3, at least in the Netherlands, plays a limited role in the adaptation of LA-MRSA to the human host.

Previous studies have shown that carriage of LA-MRSA is strongly associated with working in livestock farms. The Gaussian age distribution of people carrying LA-MRSA who reported livestock contact with most of the isolates from people aged between 21–70 years, i.e. the period when most people have an active working career, corroborates this association. The age distribution curve in people who reported not to have contact

Figure 7

Age distribution among people carrying LA-MRSA and non-LA-MRSA, the Netherlands, 2003–14



LA-MRSA: livestock-associated meticillin-resistant Staphylococcus aureus.

with livestock was flatter and had a dip in the age group 11–20 years similar to the dip in the non-LA-MRSA curve. Furthermore, the proportion of infection-related isolates in those who reported livestock contact was lower than in those reporting not to have contact with livestock. In the latter group, this proportion increased with age. The reasons for this remarkable difference in apparent pathogenicity remain unclear. However, it suggests that LA-MRSA are becoming more adapted to humans and start to resemble non-LA-MRSA in transmissibility and pathogenicity.

Our study has a number of limitations. First, all MRSA isolates originated from humans, limiting a comparison between LA-MRSA obtained from animals and humans. Second, we do not know if the question regarding animal contact in the available questionnaires was answered correctly. It could be that patients failed to remember livestock contact or misinterpreted the question and answered not having livestock contact. Third, our study only used the φ3 phage as indicator for animal or human CC398 lineages. Other markers such as tetracycline resistance and canonical SNPs as reported by Stegger et al. could perhaps have provided more differentiation [24]. Finally, we grouped the MRSA isolates in different material classes. However, it is uncertain whether isolates obtained from infection-related materials really caused MRSA infections. For instance, LA-MRSA positive sputum samples could also be the result of a contamination of the sputum sample due to carriage with LA-MRSA in the bacterial flora of the throat.

In conclusion, the emergence of a LA-MRSA subclade transmitted without livestock contact could have important implications for management strategies to control

MRSA in healthcare settings. Possible future adaptations in for instance virulence of LA-MRSA could be unnoticed for prolonged periods if different strategies are used. Therefore, careful monitoring of the different LA-MRSA MC398 types through the national MRSA surveillance and a uniform search and destroy policy regardless which MRSA variant, remains necessary.

Acknowledgements

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Conflict of interest

None declared.

Authors' contributions

TB was involved in the study design, literature search, data analysis, data interpretation, and writing of the paper. MVL was involved in the data collection, data analysis, and the bioinformatic analysis of the next generation sequencing data. GNP, FL and NMW were involved in the data collection and data analysis. SW and HGJH did the bioinformatics of the next generation sequencing data. DF and AH provided the questionnaires and were involved in the analysis of the epidemiological data. LMS supervised and managed the study and designed the primers for the φ3 specific PCR. All authors approved the final version of the manuscript.

References

1. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9(8):978-84. DOI: 10.3201/eid0908.030089 PMID: 12967497
2. Armand-Lefevre L, Ruimy R, Andremont A. Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs.Emerg Infect Dis. 2005;11(5):711-4. DOI: 10.3201/eid1105.040866 PMID: 15890125
3. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant Staphylococcus aureus in pig farming.Emerg Infect Dis. 2005;11(12):1965-6. DOI: 10.3201/eid1112.050428 PMID: 16485492
4. Bhat M, Dumortier C, Taylor BS, Miller M, Vasquez G, Yunen J, et al. Staphylococcus aureus ST398, New York City and Dominican Republic. Emerg Infect Dis. 2009;15(2):285-7. DOI: 10.3201/eid1502.080609 PMID: 19193274
5. van Cleef BA, Monnet DL, Voss A, Krziwanek K, Allerberger F, Struelens M, et al. Livestock-associated methicillin-resistant Staphylococcus aureus in humans, Europe. Emerg Infect Dis. 2011;17(3):502-5. DOI: 10.3201/eid1703.101036 PMID: 21392444
6. Graveland H, Wagenaar JA, Verstappen KM, Oosting-van Schothorst I, Heederik DJ, Bos ME. Dynamics of MRSA carriage in veal calves: a longitudinal field study.Prev Vet Med. 2012;107(3-4):180-6. DOI: 10.1016/j.prevetmed.2012.06.006 PMID: 22776914
7. Nemati M, Hermans K, Lipinska U, Denis O, Deplano A, Struelens M, et al. Antimicrobial resistance of old and recent Staphylococcus aureus isolates from poultry: first detection of livestock-associated methicillin-resistant strain ST398. Antimicrob Agents Chemother. 2008;52(10):3817-9. DOI: 10.1128/AAC.00613-08 PMID: 18663024
8. Huijsdens XW, Bosch T, van Santen-Verheuvel MG, Spalburg E, Pluister GN, van Luit M, et al. Molecular characterisation of PFGE non-typable methicillin-resistant Staphylococcus aureus in The Netherlands, 2007. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2009;14(38).
9. Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, et al. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob. 2006;5(1):26. DOI: 10.1186/1476-0711-5-26 PMID: 17096847
10. Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant Staphylococcus aureus (ST398) in Dutch hospitals. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2011;17(2):316-9.
11. McCarthy AJ, van Wamel W, Vandendriessche S, Larsen J, Denis O, Garcia-Graells C, et al. Staphylococcus aureus CC398 clade associated with human-to-human transmission. Appl Environ Microbiol. 2012;78(24):8845-8. DOI: 10.1128/AEM.02398-12 PMID: 23042163
12. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. Staphylococcus aureus CC398: host adaptation and emergence of methicillin resistance in livestock. MBio. 2012;3(1):e00305-11. DOI: 10.1128/mBio.00305-11 PMID: 22354957
13. Foster TJ. Immune evasion by staphylococci.Nat Rev Microbiol. 2005;3(12):948-58. DOI: 10.1038/nrmicro1289 PMID: 16322743
14. Bosch T, Verkade E, van Luit M, Pot B, Vauterin P, Burggrave R, et al. High Resolution Typing by Whole Genome Mapping Enables Discrimination of LA-MRSA (CC398) Strains and Identification of Transmission Events. PLoS One. 2013;8(6):e66493. DOI: 10.1371/journal.pone.0066493 PMID: 23805225
15. Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol. 2003;41(12):5442-8. DOI: 10.1128/JCM.41.12.5442-5448.2003 PMID: 14662923
16. Schouls LM, Spalburg EC, van Luit M, Huijsdens XW, Pluister GN, van Santen-Verheuvel MG, et al. Multiple-locus variable number tandem repeat analysis of Staphylococcus aureus: comparison with pulsed-field gel electrophoresis and spa-typing. PLoS One. 2009;4(4):e5082. DOI: 10.1371/journal.pone.0005082 PMID: 19343175
17. Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms.J Clin Microbiol. 2001;39(11):4190-2. DOI: 10.1128/JCM.39.11.4190-4192.2001 PMID: 11682558
18. Simpson EH. Measurement of species diversity.Nature. 1949;163(4148):688. DOI: 10.1038/163688a0
19. UC Davis School of Vet Med. 100k Genome Project [Internet] Davis: available from www.100kgenome.vetmed.ucdavis.edu
20. Statistics Netherlands [Internet]. [Accessed 21 Jul 2015]. Dutch. Available from: statline.cbs.nl
21. Lekkerkerk WS, van de Sande-Bruinsma N, van der Sande MA, Tjon ATA, Groenheide A, Haenen A, et al. Emergence of MRSA of unknown origin in the Netherlands. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012;18(7):656-61.
22. van Rijen MM, Bosch T, Verkade EJ, Schouls L, Kluytmans JA, CAM Study Group. Livestock-associated MRSA carriage in patients without direct contact with livestock.PLoS One. 2014;9(6):e100294. DOI: 10.1371/journal.pone.0100294 PMID: 25000521
23. McCarthy AJ, Witney AA, Gould KA, Moodley A, Guardabassi L, Voss A, et al. The distribution of mobile genetic elements (MGEs) in MRSA CC398 is associated with both host and country. Genome Biol Evol. 2011;3(0):1164-74. DOI: 10.1093/gbe/evr092 PMID: 21920902
24. Stegger M, Liu CM, Larsen J, Soldanova K, Aziz M, Contente-Cuomo T, et al. Rapid differentiation between livestock-associated and livestock-independent Staphylococcus aureus CC398 clades. PLoS One. 2013;8(11):e79645. DOI: 10.1371/journal.pone.0079645 PMID: 24244535

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Letter to the editor: Specificity of Zika virus ELISA: interference with malaria

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To the editor: We read the study by Huzly et al. [1] with interest and agree on the high specificity of the Euroimmun Zika virus (ZIKV) ELISA (Euroimmun, Lübeck, Germany).

We evaluated the specificity of this test on convalescent samples of 10 PCR-confirmed dengue patients (n = 3 DENV-1, n = 4 DENV-2, n = 2 DENV-3 and n = 1 DENV-4) with high IgM antibody ratios and a positive (n = 9) or negative (n = 1) result for IgG antibodies. We also tested the assay on 10 samples with high titres of neutralising antibodies against yellow fever virus and on five samples positive for rheumatoid factor. Except for one borderline result (ratio between 0.8 and 1.1) of ZIKV IgM in the convalescent sample from a patient infected with DENV-1 after a stay in Thailand, all results for ZIKV IgM and IgG were negative.

However, when we tested samples from malaria patients with a current infection (thick smear and PCR-positive) with *Plasmodium falciparum* (n = 12), *P. falciparum/P. ovale* (n = 1), *P. vivax* (n = 3), *P. ovale* (n = 5) or *P. malariae* (n = 5), or a recently treated *P. falciparum* infection (microscopy-negative, PCR-positive) (n = 8), 14 of these 34 samples tested positive or borderline for ZIKV IgM, IgG or both. Positive or borderline results for both ZIKV IgM and IgG were registered in two of 13 samples from patients with a current infection with *P. falciparum* (including the patient with the mixed *P. falciparum/P. ovale* infection) and in one of eight samples from patients with a recently treated *P. falciparum* infection. Nine samples tested positive or borderline for ZIKV IgM only: four from patients with a current *P. falciparum*, two each from patients with a *P. vivax* and recently treated *P. falciparum* infection, and one from a patient with a *P. malariae* infection. Finally, one of 13 samples from patients with a current *P. falciparum* infection and one of five samples from patients with a *P. ovale* infection tested positive for ZIKV IgG only.

Virus neutralisation tests could not demonstrate a ZIKV infection in 11 of the 14 samples with positive or borderline results. Notably, we confirmed a recent ZIKV infection in one patient with a recently treated *P. falciparum* infection who had travelled in several African countries in the first half of 2015 and most recently in Cameroon (ZIKV IgM and IgG ratios in the ELISA were 2.61 and 7.50 respectively). The results from two patients with *P. ovale* (ZikV IgG ratio: 1.84) and *P. vivax* (ZIKV IgM ratio: 0.87) infection were not conclusive. The samples from patients with a current or recently treated *P. falciparum* infection with false positive ZIKV ELISA results showed ratios between 1.10 and 6.93 for ZIKV IgM and between 0.92 and 7.02 for IgG. One sample from a patient with a *P. vivax* infection tested borderline for ZIKV IgM. The ELISA ratio for ZIKV IgM in the sample from the patient with a *P. malariae* infection was 1.10.

False positive results were not correlated with parasite densities.

Plasmodium is known for its ability to trigger polyclonal B-cell activation resulting in the production of antibodies that are not microorganism-specific [2], possibly leading to false positive results in serological assays. Samples from malaria patients should therefore be included in panels used to evaluate the specificity of assays, particularly those detecting antibodies against tropical diseases.

Although the risk for malaria in South and Central America is limited and false positive results in the ZIKV ELISA due to *Plasmodium* infections may not pose a large problem in that part of the world, species distribution modelling has shown environmental suitability for ZIKV in a large part of tropical and sub-tropical regions including Africa where malaria is endemic [3]. According to the information available for our patient with a confirmed infection, active transmission of ZIKV may occur in Africa. We therefore believe that users

of the ZIKV ELISA should be aware of the possible interference.

Conflict of interest

None declared.

Authors' contributions

KM conceived the study, KM, DVDB and MVE analysed the data, JM and KA performed and analysed neutralisation tests, MVE and DVDB wrote the manuscript.

References

1. Huzly D, Hanselmann I, Schmidt-Chanasit J, Panning M. High specificity of a novel Zika virus ELISA in European patients after exposure to different flaviviruses.Euro Surveill. 2016;21(16):30203. DOI: 10.2807/1560-7917.ES.2016.21.16.30203 PMID: 27126052
2. Scholzen A, Sauerwein RW. How malaria modulates memory: activation and dysregulation of B cells in Plasmodium infection.Trends Parasitol. 2013;29(5):252-62. DOI: 10.1016/j.pt.2013.03.002 PMID: 23562778
3. Messina JP, Kraemer MU, Brady OJ, Pigott DM, Shearer FM, Weiss DJ, et al. Mapping global environmental suitability for Zika virus. Elife. 2016;5(5):e15272.PMID: 27090089

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