Impact factor 4.659

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 19 | Weekly issue 49 | 11 December 2014

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Management of pregnant women infected with Ebola virus in a treatment centre in Guinea, June 2014

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Citation style for this article:
Baggi FM, Taybi A, Kurth A, Van Herp M, Di Caro A, Wölfel R, Günther S, Decroo T, Declerck H, Jonckheere S. Management of pregnant women infected with Ebola virus in a treatment centre in Guinea, June 2014. Euro Surveill. 2014;19(49):pii=20983. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20983

Article submitted on 25 November 2014 / published on 11 December 2014

We report two cases of confirmed Ebola virus disease in pregnant women, who presented at the Médecins Sans Frontières Ebola treatment centre in Guéckédou. Despite the very high risk of death, both pregnant women survived. In both cases the critical decision was made to induce vaginal delivery. We raise a number of considerations regarding the management of Ebola virus-infected pregnant women, including the place of amniocentesis and induced delivery, and whether certain invasive medical acts are justified.

We report two cases of confirmed Ebola virus disease (EVD) in pregnant patients who presented and were treated at the Médecins Sans Frontières (MSF) Ebola treatment centre in Guéckedou. We also raise a number of considerations regarding the role of amniocentesis and induced delivery in the management of pregnant women with EVD.

Description of the cases

Case one

Initial presentation

At the beginning of June 2014, a woman in her late 20s at seven months gestation presented at the Ebola treatment centre in Guékedou, Guinea, with a history of seven days of asthenia, fever (self-reported), and vomiting. Her past obstetrical history included six vaginal deliveries and no abortions. On admission, physical examination revealed a temperature of 37.1 °C, mild dehydration and the patient reported fetal movement. The Ebola virus (EBV) test (real-time reverse transcription-polymerase chain reaction (RT-PCR)) was positive.

Clinical course and management

On the same day (day 0), the woman was admitted to the EBV treatment/isolation unit where she immediately started receiving supportive treatment, including Ringer's lactate, antipyretics, ceftriaxone (2 g/day), metoclopramide and omeprazole. The woman responded well to this supportive treatment and by day six of her admission, she was free from symptoms, and reported continuously fetal movements. On day eight and 10, the results of the EBV tests came back negative, and the woman was considered cured. The woman remained in the unit for further monitoring.

On day 11, the woman's temperature rose to 38 °C, and further examination revealed that fetal movements and heartbeat had stopped. Cervical examination showed no uterine contractions, no cervical dilation, no blood or other discharge. Intravenous metronidazole was added for suspected chorioamnionitis. To evaluate the possibility of maternal-to-fetal EBV transmission, an amniocentesis was performed. The clear-coloured amniotic fluid contained a high Ebola viral load (corresponding to a real-time RT-PCR cycle threshold (CT) value of 21.29).

On day 15, the patient was afebrile. An assisted delivery was organised to take place in the high-risk zone of the treatment centre. Labour induction with misoprostol resulted in a vaginal delivery of a stillborn male fetus (first degree maceration). The placenta was complete. No episiotomy was required, uterine bimanual massage, oxytocin (10 Units intravenous) and ergometrine (one vial of 0.2 mg intramuscular) helped obtaining normal uterine retraction and prevented any excessive post-partum bleeding. The samples from the placenta (maternal and fetal side), meconium, and the fetus (intra-cardiac aspiration, throat swab, ear swab, umbilical cord) were EBV positive (Table). The patient was afebrile after delivery, and was discharged on day 18. A seven-days post-natal consultation (PNC) showed a normal evolution.

Table

Test results from maternal and fetal samples taken from two pregnant patients during their stay at the Ebola treatment centre, Guéckédou, Guinea, June 2014

Timeline	Specimen type	Ebola virus load result (CT value)	Semi-quantitative viral load result	Other results
Patient 1				
Day 0 (admission)	Blood (mother)	Positive (21.29)	+ + +	Malaria negative
Day 8	Blood (mother)	Negative (-)	-	IgG positive (≥ 1:1,280) IgM positive (≥ 1:320)
Day 10	Blood (mother)	Negative (-)	-	–
Day 12	Amniotic fluid (amniocentesis)	Positive (23.31)	+ + +	–
Day 15	Amniotic fluid (fetal mouth swab)	Positive (21.41)	+ + +	–
Day 15	Amniotic fluid (fetal ear swab)	Positive (24.78)	+ + +	–
Day 15	Placenta (fetal side)	Positive (24,12)	+ + +	–
Day 15	Placenta (maternal side)	Positive (19,23)	+ + +	–
Day 15	Fetal blood – sample 1	Positive (16,13)	+ + +	–
Day 15	Fetal blood – sample 2	Positive (23.6)	+ + +	–
Day 15	Fetal meconium (anus swab)	Positive (20,32)	+ + +	–
Day 18	Blood (mother)	Negative (-)	-	–
Patient 2				
Day 1[a]	Blood (mother)	Positive (26.46)	+ +	Malaria positive
Day 7	Blood (mother)	Positive (25,43)	+ +	–
Day 11	Amniotic fluid (mouth swab)	Positive (24.10)	+ + +	–
Day 11	Amniotic fluid (fetal ear swab)	Positive (28.82)	+ +	–
Day 11	Placenta (fetal side)	Positive (14.22)	+ + +	–
Day 11	Placenta (maternal side)	Positive (19.98)	+ + +	–
Day 11	Fetal meconium (anus swab)	Negative (-)	-	–
Day 16	Blood (mother)	Negative (-)	-	–
Day 18	Blood (mother)	Negative (-)	-	–

CT: cycle threshold; NA: not available; RT-PCR: reverse transcription-polymerase chain reaction.

[a] Patient 2 first presented at the treatment centre in the afternoon (day 0) so the result of Ebola virus testing was available the next day (day 1).

Real-time RT-PCR was performed with the Smart Cycler. The obtained CT values correspond with the accumulation of the fluorescent signal and are inversely proportional with the viral load. CT values are classified in subsequent categories of 0–25, 25–35 and 35–40 and correspond with + + +, + + and + results.

When the real-time RT-PCR was negative this is indicated for the viral load by a – result.

Case two

Initial presentation

In mid-June 2014 a primipara in her early 20s at seven months gestation presented with a history of five days of arthralgia, asthenia, diarrhoea, fever (self-reported) and headache. The patient presented with a history of grade III female genital mutilation (FGM). On admission, the patient had a temperature of 38.4 °C, and reported fetal movements and no contractions.

Clinical course and management

On the next day (day 1), the EBV test and malaria rapid test were positive. The patient's fever worsened (39.5 °C), and she had an onset of haematuria and cough. The patient reported fetal movements had stopped. Supportive treatment included intravenous ampicillin and metronidazole for a possible chorioamnionitis, as well as intravenous artesunate (malaria treatment) and Ringer's lactate.

On day five, the patient's systolic blood pressure dropped to 60 mmHg (norm: 90–119) and additional fluids were intravenously administered. On day eight, the patient presented with symmetrical oedema of the lower extremities. Obstetrical examination revealed a hypertonic uterus, transverse or breech presentation, no fetal heartbeat, cervical dilation of one centimeter in diameter, and no discharge. Despite the risk, ketamine anaesthesia was provided, external version manoeuvres were performed, and the fetus was rendered in cephalic presentation.

On day 10, the patient was disoriented and presented with anasarca. On the morning of day 11, the patient was found unconscious, with the fetal head intravaginal. Ketamine was administered, an episiotomy was performed, and a male stillborn fetus was delivered vaginally. The placenta was complete. Urinary retention complicated uterine retraction, and uterine bi-manual massage was employed together with the administration of oxytocin (10 U) and ergometrine (1 vial of 0.2mg). Post-partum haemorrhage only stopped after repeatedly (five times) packing the uterus with gauze. Due to the FGM, bladder catheterisation was unsuccessful. Urine was aspirated through a supra-pubic bladder paracentesis (the urine was not tested for EBV). A final vaginal and uterine exploration showed no further complications. The samples from the placenta (maternal and fetal side) and the fetus (throat swab, ear swab) were EBV positive. The sample from the meconium was negative (Table). No pericardial puncture was performed.

On day 12, the patient regained consciousness, and spontaneous diuresis resumed after a single dose dexamethasone injection. The patient had a temperature of 40 °C. Gentamicin was added to the treatment. Over the next six days the patient improved clinically. On day 17, the patient was afebrile. On day 16 and 18, EBV tests were negative and the patient was considered cured. The patient was discharged on day 19. She did not attend her scheduled appointment seven-days PNC.

Ebola virus outbreak in West Africa

In March 2014, an EVD outbreak was declared in Guéckedou, Guinea, following which it spread to Liberia, Sierra Leone, Nigeria, Senegal and Mali [1,2]. The viral strain responsible for the current outbreak has been identified as the Zaire strain, a particularly virulent strain associated with mortality rates as high as 90% [1]. Overall, by 21 November 2014, 15,351 individuals have become infected and 5,459 of these have died. Among those infected, 588 were healthcare workers and 377 of these have died [2]. Patients with EVD generally present with a history of contact with another person with EVD and an abrupt onset of a non-specific febrile syndrome. A systemic inflammatory response can cause multiple organ failure and shock [3,4]. Pregnant women are reported to be at higher risk to die [5].

Since the onset of the outbreak in Guinea, MSF has set up and is running six Ebola treatment centres – including one in Guékédou where the outbreak began.

Discussion

There are very few studies reporting on maternal and fetal outcomes of pregnant women infected with EBV. We report on two cases of pregnant women infected with EBV in Guinea. Despite pregnant women being at higher risk of more severe disease and mortality [5], both women survived. Both fetuses unfortunately died in utero. This case report raises a number of important points for discussion regarding the management of pregnant women infected with EBV.

Although our findings are based on two cases only, they depict a more positive picture of the maternal outcomes of EVD during pregnancy. In both cases the delivery occurred during the healing phase, when the EBV viraemia in the pregnant woman was controlled, and when clotting had probably returned to normal. During previous outbreaks also caused by the Zaire strain, such as in the Democratic Republic of the Congo (DRC) 1976 Yambuku outbreak, only nine (11%) of 82 EBV infected pregnant women were reported to have survived [6]. Similarly, during another EBV outbreak in the DRC 20 years later (1995 in Kikwit) only one (7%) of 15 pregnant women was reported to have survived, and EBV-infected pregnant women had a notably higher mortality rate (93%) than non-pregnant EBV-infected women (70%) [5]. For the current outbreak, data on pregnancy are not routinely reported so overall figures on the survival of pregnant women and their unborn children or neonates are not available at this point.

Despite the two women described in this report surviving, in both cases the fetus died in utero. There is not much chance for the fetus to survive EBV infection. A massive infection of the fetus is likely to occur through the placenta. Furthermore maternal immunoglobulins M are poorly transported through placental villi and the fetal secretory immune system starts producing immunoglobulin M around the 20th week of gestation [7]. Fetal and neonate mortality was equally reported high in other outbreaks. In Yambuku, 11 live neonates were born to EBV-infected women and all died within 19 days [6]. In Yambuku and Kikwit, abortion occurred among 19 of 82 (23%) and 10 of 15 (67%) infected pregnant women respectively [5,6]. In Kikwit the only surviving pregnant patient had an abortion [5]. In Yambuku, one of the nine survivors aborted in the treatment centre. Abortions occurred spontaneously, likely because of fetal death due to EBV infection. Unfortunately the pregnancy outcomes of the other pregnant EVD survivors were not reported [6].

This is the first description of the use of amniocentesis to determine the presence of intrauterine EBV infection. In the case of the first patient, despite her having an undetectable viral load and declared cured before the demise of the fetus, a subsequent amniocentesis revealed a high viral load in the amniotic fluid. For this reason, a vaginal delivery was arranged to take place in the high-risk zone of the EVD treatment centre. In the absence of the amniocentesis, the recovered EBV negative patient might have been referred to the local maternity for delivery, exposing the maternity staff to a very high risk of EBV infection. Alternatively, she might have had a spontaneous abortion at home with potential risk of subsequent transmission of EBV to household contacts. In the second case, an emergency

delivery was required and performed at the EVD treatment centre after the patient was found in shock, with intra-vaginal fetal head. This delivery also took place in the high-risk zone of the centre and episiotomy was justified due to the grade III FGM. Post-partum, an amniotic fluid sample was taken from the dead fetus through an oral swab and yielded a high EBV viral load.

For both cases, the assisted delivery occurred a few days after fetal movement had reportedly stopped. As these were the first induced deliveries of EBV infected pregnant women, careful planning had to be considered as well precautionary measures, given the high risk of nosocomial transmission to healthcare workers [3]. Moreover, based on previous reports [5,6], it was also taken into account that spontaneous abortions could occur shortly after fetal death, limiting the need for invasive procedures, and reducing the risk to healthcare staff.

During both deliveries, strict barrier nursing techniques were used. Full protective equipment included scrubs, waterproof overall, apron, boots, N95 masks, head cover, goggles, a double pair of gloves and arm-length gynaecological gloves (three layers of gloving). Absorbent pads were laid underneath the patients to absorb a maximal amount of fluids. Two pads were laid over the abdomen and the perineal region to limit splashing. Biomedical waste was gathered in the immediate proximity of the patient, and regularly sprayed with 0.5% chlorine solution. None of the five healthcare workers who were present during the deliveries reported here became infected.

These two case presentations raise a number of considerations regarding the management of pregnant women infected with EBV including the role of amniocentesis and induced delivery, and whether certain invasive medical procedures are justified, despite the inherent risk for healthcare workers.

In conclusion, our case report adds to the scarce body of literature on the outcomes of pregnant women infected with EBV. We also highlight some important considerations in the management of such patients and describe, for the first time, the use of amniocentesis to detect fetal infection with EBV.

Acknowledgements

We would like to thank Marguerite Ouamouno, Pauline Yombouno, Adèle Millimouno and all MSF staff from Guékédou for their support in taking care of these and many other patients. We acknowledge Rosa Crestani and Katherine Harries for their critical review of the manuscript.

Conflict of interest

None declared.

Authors' contribution

Fernanda Mendez Baggi, Aicha Taybi, Andreas Kurth, and Sylvie Jonckheere collected data in the MSF Ebola treatment centre in Guéckedou, and wrote the first draft. Michel Van Herp, Antonino Di Caro, Roman Wölfel, Stephan Günther, Hilde Declerck, and Tom Decroo all reviewed the first draft and final version of the paper, and their comments were incorporated.

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Emergence of clonally related multidrug resistant *Haemophilus influenzae* with penicillin-binding protein 3-mediated resistance to extended-spectrum cephalosporins, Norway, 2006 to 2013

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Citation style for this article:
Skaare D, Anthonisen IL, Kahlmeter G, Matuschek E, Natås OB, Steinbakk M, Sundsfjord A, Kristiansen BE. Emergence of clonally related multidrug resistant Haemophilus influenzae with penicillin-binding protein 3-mediated resistance to extended-spectrum cephalosporins, Norway, 2006 to 2013. Euro Surveill. 2014;19(49):pii=20986. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20986

Article submitted on 07 May 2014 / published on 11 December 2014

Resistance to cephalosporins in *Haemophilus influenzae* is usually caused by characteristic alterations in penicillin-binding protein 3 (PBP3), encoded by the *ftsI* gene. Resistance to extended-spectrum cephalosporins is associated with high-level PBP3-mediated resistance (high-rPBP3), defined by the second stage S385T substitution in addition to a first stage substitution (R517H or N526K). The third stage L389F substitution is present in some high-rPBP3 strains. High-rPBP3 *H. influenzae* are considered rare outside Japan and Korea. In this study, 30 high-rPBP3 isolates from Norway, collected between 2006 and 2013, were examined by serotyping, multilocus sequence typing (MLST), *ftsI* sequencing, detection of beta-lactamase genes and minimum inhibitory concentration (MIC) determination. MICs were interpreted according to clinical breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Respiratory isolates predominated (proportion: 24/30). The 30 isolates included one serotype f isolate, while the remaining 29 lacked polysaccharide capsule genes. Resistance to extended-spectrum cephalosporins (cefixime, 29 isolates/30 isolates; cefepime, 28/30; cefotaxime, 26 /30; ceftaroline, 26/30; ceftriaxone, 14/30), beta-lactamase production (11/30) and co-resistance to non-beta-lactams (trimethoprim-sulfamethoxazole, 13/30; tetracycline, 4/30; chloramphenicol, 4/30; ciprofloxacin, 3/30) was frequent. The N526K substitution in PBP3 was present in 23 of 30 isolates; these included a blood isolate which represents the first invasive S385T+N526K isolate reported from Europe. The L389F substitution, present in 16 of 30 isolates, coincided with higher beta-lactam MICs. Non-susceptibility to meropenem was frequent in S385T+L389F+N526K isolates (8/12). All 11 beta-lactamase positive isolates were TEM-1. Five clonal groups of two to 10 isolates with identical MLST-*ftsI* allelic profiles were observed, including the first reported high-rPBP3 clone with TEM-1 beta-lactamase and co-resistance to ciprofloxacin, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole. Prior to this study, no multidrug resistant high-rPBP3 *H. influenzae* had been reported in Norway. Intensified surveillance of antimicrobial resistance is needed to guide empiric therapy.

Introduction

Haemophilus influenzae colonises the respiratory tract in humans and infection causes a wide range of conditions, including acute otitis media, arthritis, conjunctivitis, epiglottitis, meningitis, respiratory tract infections, septicaemia and sinusitis [1]. Encapsulated isolates of serotype b (Hib) have the highest potential for invasive disease but the introduction of Hib vaccines has dramatically reduced the burden of disease. Thus, the vast majority of infections, both invasive and non-invasive, are currently caused by nontypeable (i.e. lacking polysaccharide capsule) *H. influenzae* (NTHi) and non-b serotypes, mainly serotype f (Hif) [1-3].

Due to the global emergence of beta-lactamase (TEM-1 and ROB-1) producing strains in the 1970s, empiric therapy for severe disease caused by *H. influenzae* was altered from ampicillin to cephalosporins [4]. Development of resistance to extended-spectrum cephalosporins has forced a second shift in empiric treatment to carbapenems in some geographical areas [4]. The European Committee on Antimicrobial

Susceptibility Testing (EUCAST) categorises resistance to extended-spectrum cephalosporins in *H. influenzae* as an exceptional phenotype [5]. Considering such isolates 'very rare or not yet reported', EUCAST recommend that they are sent to a reference laboratory [6]. Notably, minimum inhibitory concentration (MIC) breakpoints for cephalosporins and *H. influenzae* from EUCAST are considerably lower than those from Clinical and Laboratory Standards Institute (CLSI): for instance, the susceptible minimum inhibitory concentration (MIC) breakpoint for cefotaxime from EUCAST is ≤ 0.12 mg/L while the corresponding CLSI breakpoint is ≤ 2 µg/mL [7].

Resistance to cephalosporins is due to alterations in the transpeptidase domain of penicillin-binding protein 3 (PBP3), encoded by the *ftsI* gene, with phenotypic resistance profiles depending on amino acid substitution patterns. Several genotypic classification systems for PBP3-mediated resistance (rPBP3) have been suggested and the terminology is still developing [4,8-13]. Acquirement of one of the substitutions R517H or N526K represents the first stage of resistance development. The second stage substitution S385T is associated with increased resistance to cefotaxime and separates high-level resistant (high-rPBP3) isolates from those with low-level resistance (low-rPBP3) [4,12,13]. High-rPBP3 strains may be divided into group III (S385T + N526K) and group III-like (S385T + R517H) [9-11]. The additional L389F substitution is associated with further increased resistance levels in high-rPBP3 strains and may be considered the third stage in development of PBP3-mediated resistance [13].

High-rPBP3 isolates became frequent in Japan in the late 1990s [14] and in Korea during the 2000s [15]. A similar development in other geographical areas would compromise current empiric antimicrobial therapy in severe infections, which is largely based on third-generation cephalosporins. However, low-rPBP3 *H. influenzae*, lacking the S385T substitution and mainly susceptible to cefotaxime and ceftriaxone, still predominate in south-west and northern Europe [2,8,11,16]. In previous systematic surveys outside Asia where determination of PBP3 amino acid substitution patterns has been performed [2,3,8,11,16-19], very few group III isolates have been reported [1]; these include one invasive isolate from Canada [19], one otitis media isolate from France [16] and one upper respiratory tract isolate from Norway [8]. Group III-like isolates have been more frequently reported in Europe [1,3,8,11,16,18].

Since 2007, the Norwegian Working Group on Antibiotics (NWGA) has recommended that cefotaxime resistant *H. influenzae* isolates are sent to the reference laboratory for *ftsI* sequencing and susceptibility testing with broth microdilution. In Norway, this is carried out at Vestfold Hospital Trust, in collaboration with the Haemophilus Reference Laboratory at the Norwegian Institute of Public Health and the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance at the University Hospital of North Norway. We present here susceptibility profiles, resistance genotypes and molecular strain characteristics of 30 high-rPBP3 isolates from Norway, defined by the S385T substitution, collected between May 2006 and July 2013.

Methods

Isolates

A total of 30 clinical high-rPBP3 (S385T positive) *H. influenzae* isolates from Norway, isolated at 10 different routine laboratories between May 2006 and July 2013, were included in the study. Of the 30 high-rPBP3 isolates, 27 (from 7 laboratories) with the S385T substitution were identified by partial *ftsI* sequencing of 39 routine isolates (from 10 laboratories) with cefotaxime MIC > 0.12 mg/L (gradient test); the remaining 12 isolates were categorised as group II low-rPBP3 (N526K positive, S385T negative) and had cefotaxime MIC = 0.25 mg/L (n = 10) or 0.5 mg/L (n = 2) by gradient test. We also included three isolates (isolates 4–6) from three additional laboratories, collected in 2007 and identified as high-rPBP3 through a previous surveillance study [8]. The 10 laboratories contributing the 30 isolates were located in mid Norway (Nord-Trøndelag county), the Oslo region (Akershus, Buskerud, Oslo and Vestfold counties), Western Norway (Sogn og Fjordane, Hordaland and Rogaland counties) and Southern Norway (Agder county).

Pure cultures were kept frozen at -70 °C in Microbank vials (Pro-Laboratory Diagnostics, Ontario, Canada) pending further characterisation.

Demographic and clinical data

Information about sample types, demographic data and brief clinical information (provided by the clinician at the time of sampling) were acquired from the primary laboratories. Three recently hospitalised patients with X-ray confirmed pneumonia most likely caused by study isolates were selected as case reports. For these patients, relevant supplementary information about diagnosis, antimicrobial therapy and clinical outcome was extracted from the patient files.

Species identification and molecular characterisation

Species identification was carried out by API NH (bioMérieux, Marcy-l'Etoile, France), matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry with MALDI Biotyper (Bruker Daltonics GmbH, Bremen, Germany), and conventional methods, including absence of beta-haemolysis on blood agar. Amplification of the *fucK* gene when performing multilocus sequence typing (MLST) (see below) was used as an additional criterion to separate *H. influenzae* from H. haemolyticus; all included isolates were *fucK* positive.

Biotypes [20] were determined using indole, urease and ornithine decarboxylase (ODC) reactions produced by API NH (bioMérieux).

Capsular serotyping was performed at the Norwegian Institute of Public Health according to previously described methodology [21] with modifications (underscored) to the e1 (TTTGGTAACGAATGTAGTGGTAG) and e2 (ATAGCTTTACTGTATAAGTCTTAG) primers (5´ to 3´).

MLST with sequencing of the seven housekeeping genes *adk, atpG, frdB, fucK, mdh, pgi* and *recA* was carried out at the Norwegian Institute of Public Health according to standard procedures [22]. Sequences were registered at http://haemophilus.mlst.net. Sequence types (ST) were assigned according to allelic profiles. STs were divided into clonal complexes (CC) (named according to founder) using eBURST Version 3 (http://haemophilus.mlst.net/eburst).

Pulsed-field gel electrophoresis (PFGE) of selected isolates was carried out at Unilabs Telelab (Skien, Norway) as previously described [17] and clonal relatedness was interpreted according to the Tenover criteria [23].

Beta-lactamase detection

All isolates were screened for beta-lactamase production and the presence of beta-lactamase genes (TEM-1 and ROB-1) as previously described using acidimetric agar [24] and polymerase chain reaction (PCR) [17].

Minimum inhibitory concentration determination

MICs to ampicillin, amoxicillin (ampicillin-sulbactam 2:1 ratio and amoxicillin-clavulanic acid 2:1 ratio for beta-lactamase positive isolates), cefuroxime, cefixime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, ciprofloxacin, levofloxacin, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole were determined by broth microdilution according to CLSI methodology [25] at Vestfold Hospital Trust, using standard Sensititre plates (HPB1, STP6 and GNX3F; TREK Diagnostic Systems, Thermo Fisher Scientific, West Sussex, UK). Ceftaroline, azithromycin, clarithromycin, erythromycin, roxithromycin, telithromycin, doxycycline, minocycline and rifampicin MICs were determined at the EUCAST Development Laboratory, using custom Sensititre plates (SEFE3, TREK) with in-house prepared EUCAST MH-F broth (cation-adjusted Mueller-Hinton broth (BBL, BD Diagnostic Systems, Franklin Lakes, NJ) with 5% lysed horse blood and 20 mg/L beta-NAD) and 16 to 20 hours incubation in sealed panels in ambient air. *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766 and *H. influenzae* ATCC 35056 (ampicillin-sulbactam and amoxicillin-clavulanic acid only) were incorporated for quality control purposes, with all MICs within accepted ranges. MICs were interpreted according to EUCAST clinical breakpoints [6]. MICs of all study isolates are included in the EUCAST MIC database (www.eucast.org/MIC_distributions).

ftsI/penicillin-binding protein 3 typing

PCR of the *ftsI* gene (transpeptidase domain) was performed as described previously [17]. Sequencing was performed at GATC Biotech AG (Konstanz, Germany). Sequences were analysed with the Lasergene software (DNASTAR, Madison, WI, US). An unweighted pair group method with arithmetic mean (UPGMA) phylogram (nucleotides 1010–1719) was created using ClustalW2 (www.ebi.ac.uk) and TreeDyn (www.phylogeny.fr). *H. influenzae* Rd KW20 (GenBank accession number: cU32793) was used as reference and *H. parainfluenzae* (European Molecular Biology Laboratory (EMBL) accession number: AB267856) as outgroup. The 710 bp fragments (hereafter denoted *ftsI* alleles) were assigned numbers (1–10) according to frequency of occurrence and used together with MLST allelic profiles for assignment to clonal groups (CG) (see below).

Deduced PBP3 amino acid sequences (338–573) were compared with *H. influenzae* Rd KW20 and sequence patterns (denoted PBP3 types) were assigned numbers (1–7) according to frequency of occurrence. The substitutions S385T, R517H and N526K were used for categorisation into PBP3 group III and group III-like [9-11]. For isolates with the third stage substitution L389F, the suffix '+' was added. The following group designations were thus used: group III (S385T, N526K); group III+ (S385T, L389F, N526K); group III-like (S385T, R517H); group III-like+(S385T, L389F, R517H). The groups respectively correspond to PBP3 classes II, III, V and VI, proposed by Osaki et al. [13].

Clonal groups

According to a previously described approach to molecular typing of *H. influenzae* [4], CGs were defined as isolates with identical MLST-*ftsI* allelic profiles and numbered chronologically [1-5].

GyrA and *ParC* amino acid substitutions

For isolates with ciprofloxacin MIC›0.06 mg/L and/or levofloxacin MIC›0.03 mg/L, the quinolone-resistance determining regions (QRDR) of *gyrA* and *parC* were characterised by PCR methodology adapted from a previous report [26]. DNA sequencing of amplified products was carried out at GATC Biotech. Sequences were analysed with Lasergene. Deduced amino acid sequences of *GyrA* (64–177) and ParC (66–236) were compared with *H. influenzae* Rd KW20 (GenBank accession number: U32793).

Nucleotide sequences

The *ftsI* (HG983286–HG983315), *gyrA* (HG983316–HG983320) and *parC* (HG983321–HG983325) sequences from this study are available at the European Nucleotide Archive (www.ebi.ac.uk).

Ethics

The use of clinical data in this study is approved by the Regional Committees for Medical and Health Research Ethics in Norway (reference number 2014/411).

Table 1

Characterisation of *Haemophilus influenzae* with high-level penicillin-binding protein 3-mediated beta-lactam resistance (high-rPBP3), Norway, 2006–2013 (n=30)

Isolate[a]	Year/ region[b]		Category[c]/ age group[d]		Site[e]	Clinical data[f]	Biotype[g]	MLST		*ftsI*[h]	Clonal group[i]	PBP3		Co-resistance[l]
								ST	CC			Type[j]	Group[k]	
1	2006	O	Hosp	1	Nose	ALL/AOM	III	155	155	3	1	4	III-like	–
2	2007	O	GP	1	Nose	URTI	IV	1197	395	1	2	1	III	β
3	2007	O	GP	5	Npx	No data	III	159	503	6	Single	5	III+	Ts
4	2007	O	OC	3	Nose	No data	IV	1197	395	1	2	1	III	–
5	2007	O	OC	1	Npx	No data	III	155	155	3	1	4	III-like	–
6	2007	M	Hosp	7	LRT	No data	III	155	155	3	1	4	III-like	–
7	2008	O	GP	3	Npx	No data	III	1197	395	1	2	1	III	–
8	2008	O	Hosp	1	Nose	URTI	IV	1197	395	1	2	1	III	–
9	2008	O	GP	1	Npx	No data	IV	1197	395	1	2	1	III	–
10	2008	O	GP	1	Nose	No data	IV	1197	395	1	2	1	III	–
11	2009	O	GP	5	Npx	Sinusitis	I	408	3	7	Single	6	III+	–
12	2009	W	OC	1	Ear	AOM	IV	1197	395	1	2	1	III	–
13	2009	W	GP	6	Nose	Sinusitis	III	1197	395	1	2	1	III	–
14	2009	W	GP	1	Ear	AOM	III	1197	395	1	2	1	III	–
15	2010	W	GP	1	Npx	AOM	III	1197	395	1	2	1	III	–
16	2011	O	GP	1	Eye	Conjunctivitis	II	422	422	4	3	3	III-like+	β,q,Ts
17	2011	O	GP	4	Nose	Sinusitis	II	142	142	8	Single	3	III-like+	–
18	2012	S	Hosp	8	Blood	MM/pneumonia	I	1287	None	9	Single	7	III	–
19	2012	W	OC	8	BAL	COPD/DC	II	160	160	2	Single	2	III+	Ts
20	2012	W	OC	1	Sputum	CF	III	148	245	10	Single	2	III+	–
21	2012	O	GP	1	Ear	AOM	I	124	124	2	Single	2	III+	Ts
22	2013	W	GP	8	Sputum	Pneumonia	IV	1282	503	4	Single	3	III-like+	β,C,T,Ts
23	2013	O[m]	Hosp	7	Nose	Pneumonia	II	422	422	4	3	3	III-like+	β,q,Ts
24	2013	W	Hosp	8	Eye	Dacryocystitis	III	159	503	5	4	2	III+	β,Q,C,T,Ts
25	2013	W	OC	1	Npx	PCD	III	159	503	5	4	2	III+	β,Q,C,T,Ts
26	2013	W	OC	4	Sputum	CF/pharyngitis	III	159	503	5	4	2	III+	β,Q,C,T,Ts
27	2013	W	Hosp	8	Sputum	CVD/pneumonia	IV	836	245	2	5	2	III+	β,Ts
28	2013	W	Hosp	8	Sputum	DC/pneumonia	III	836	245	2	5	2	III+	β,Ts
29	2013	W	Hosp	7	Sputum	COPD/pneumonia	IV	836	245	2	5	2	III+	β,Ts
30	2013	W	Hosp	8	Npx	Possible pneumonia	III	836	245	2	5	2	III+	β,Ts

CC: clonal complex; MLST: multilocus sequence typing; PBP3: penicillin-binding protein 3; ST: sequence type.

[a] All isolates lacked polysaccharide capsule genes except for isolate 21 (serotype f).

[b] M: mid Norway (Nord-Trøndelag); O: Oslo region (Oslo, Akershus, Buskerud, Vestfold); S: Southern Norway (Agder); W: Western Norway (Sogn og Fjordane, Hordaland, Rogaland).

[c] GP: general practice; Hosp: hospitalised; OC: outpatient clinic.

[d] 1: 0–9 years; 2: 10–19 years; 3: 20–29 years; 4: 30–39 years; 5: 40–49 years; 6: 50–59 years; 7: 60–69 years; 8: ≥70 years.

[e] BAL: bronchioalveolar lavage; LRT: lower respiratory tract; Npx: nasopharynx.

[f] ALL: acute lymphoblastic leukemia; AOM: acute otitis media; CF: cystic fibrosis; COPD: chronic obstructive pulmonary disease; CVD: cardiovascular disease; DC: disseminated cancer; MM: multimorbidity (hypogammaglobulinemia, chronic lymphocytic leukaemia, myasthenia gravis, COPD); PCD: primary ciliary dyskinesia; URTI: upper respiratory tract infection.

[g] According to reactions (positive (+) or negative (-)) to indole, urease and ornithine decarboxylase (ODC) respectively. I: +/+/+; II: +/+/-; III: -/+/-; IV: -/+/+ [18].

[h] *ftsI* alleles (Figure 1).

[i] Isolates with identical MLST-*ftsI* allelic profiles (Figure 1). Isolates qualified as 'single' are those among the 30 analysed with a unique MLST-*ftsI* allele combination.

[j] PBP3 type according to amino acid sequences (substitutions underscored) in positions 350, 357, 377, 385, 389, 502, 517, 526, 532, 547, 557, 562 and 569. 1: NNMTLTRKTIYVS; 2: NNITFARKTIYLS; 3: NNITFAHNSIHVS; 4: NNITLAHNSIYVN; 5: NNITFTRKTVYVN; 6: NNITFTRKTVYLN; 7: DSMTLTRKTIYVS.

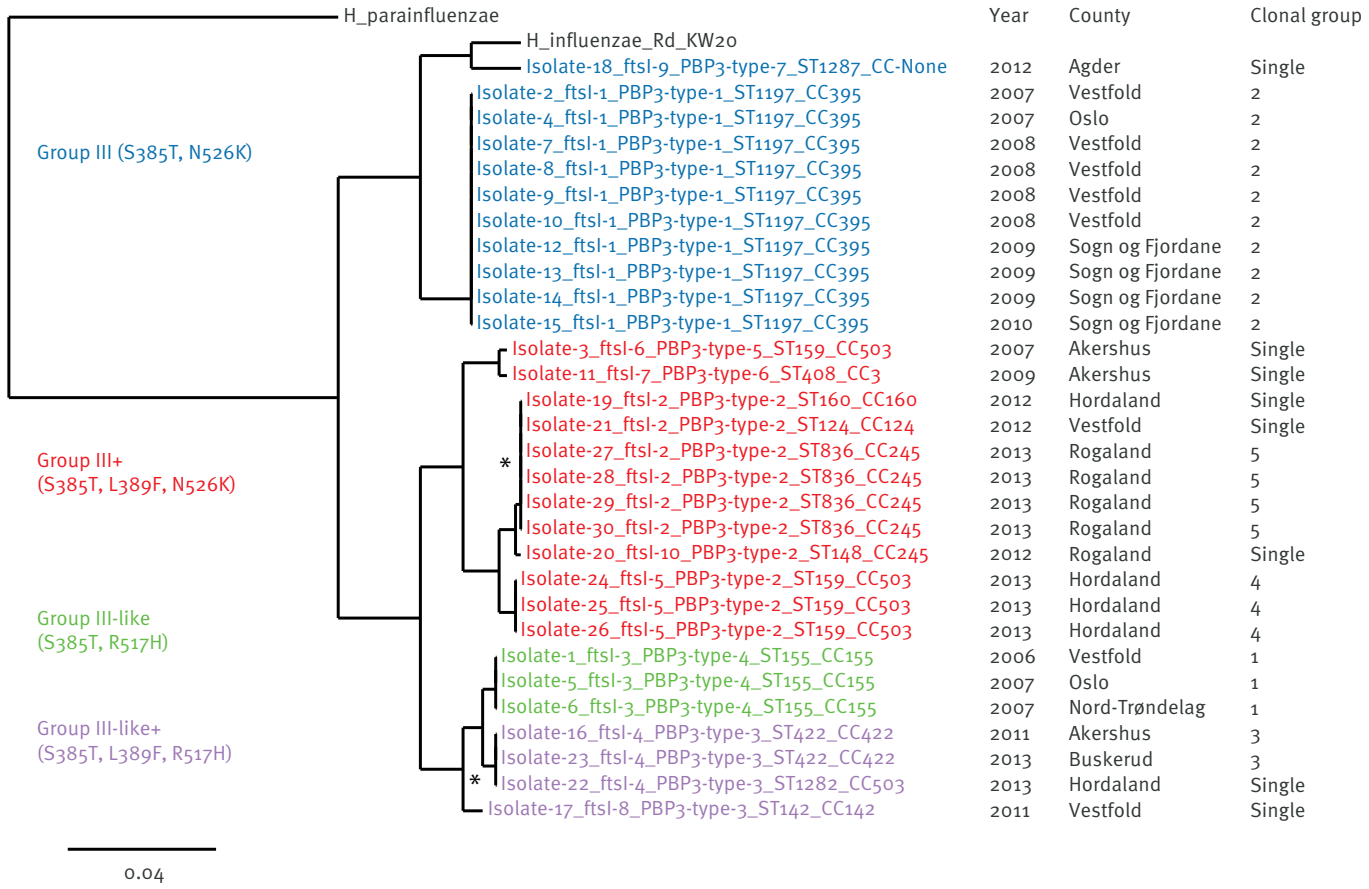
[k] Group III: S385T and N526K; group III+: S385T, L389F and N526K; group III-like: S385T and R517H; group III-like+: S385T, L389F and R517H.

[l] β: TEM-1 beta-lactamase; Q: quinolones; q: quinolones (low level resistant); C: chloramphenicol; T: tetracyclines; Ts: trimethoprim-sulfamethoxazole; –: none.

[m] Recently travelled to Thailand.

Figure 1

Phylogram of *ftsI* DNA sequences from *Haemophilus influenzae* with high-level penicillin-binding protein 3-mediated beta-lactam resistance (high-rPBP3), Norway, 2006–2013 (n=30)



The phylogram was constructed using unweighted pair group method with arithmetic mean (UPGMA) and based on *ftsI* DNA sequences (transpeptidase domain, nucleotides 1010–1719) including *H. influenza*e Rd KW20 (GenBank accession number: U32793) as reference and *H. parainfluenzae* (European Molecular Biology Laboratory (EMBL) accession number: AB267856) as outgroup.

The scale is DNA divergence.

Labels indicate isolate numbers, *ftsI* alleles, penicillin-binding protein 3 (PBP3) types (Table 1), multilocus sequence typing sequence types (ST) and clonal complexes (CC).

Colours indicate PBP3 groups. Asterisks indicate *ftsI* alleles carried by unrelated STs.

Year/county of isolation and assignment to clonal groups (CG) to the right.

Results

Patient data

The median age of the 30 patients from whom the respective isolates were derived, was 29 years (range: 0–91). In the age group zero to nine years, most patients (proportion: 10/13) were below five years-old. The male/female ratio was 13/17. All but two patients lived in the Oslo region (14/30) or in Western Norway (14/30). Except for the patient with isolate 23, recently returning from Thailand, there was no known recent (‹3 months) travel history abroad (Table 1).

Ten isolates (10/30) were from hospitalised patients. The proportion of hospitalised patients was 4/21 in the period between 2006 and 2012 compared to 6/9 in 2013. One of the 30 isolates was from blood; the rest were eye (2/30), ear (3/30) or respiratory isolates (24/30). Among the patients for whom clinical information was available (n=23), pneumonia (n=7) and acute otitis media (AOM) (n=5) were the most frequent infection types. At least seven of the 30 patients were predisposed for respiratory tract infections due to underlying chronic condition (Table 1).

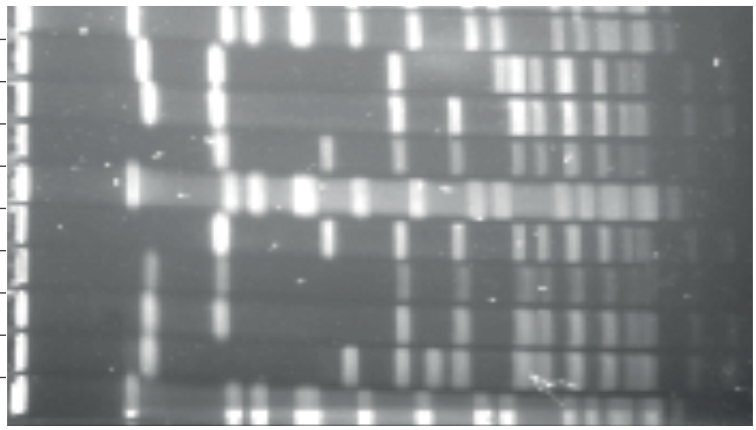
Strain characterisation

All isolates but one (Hif) lacked polysaccharide capsule genes and were categorised as nontypeable (NTHi). The isolates were biotypes I (proportion: 3/30), II (4/30), III

Figure 2

Pulsed-field gel electrophoresis (PFGE) band patterns and pulsotypes for *Haemophilus influenzae* isolates of clonal group 2, Norway, 2006–2013 (n=7)

Isolate	Year	County	Region	Pulsotype
Std				
2	2007	Ve	O	3
15	2010	SF	W	1
13	2009	SF	W	2
Std				
12	2009	SF	W	2
14	2009	SF	W	1
8	2008	Ve	O	1
4	2007	Os	O	4
Std				



O: Oslo region; Os: Oslo county; SF: Sogn og Fjordane county; Std: size standard (*Staphylococcus aureus* NCTC 8325); Ve: Vestfold county; W: Western Norway.

Labels indicate isolate numbers, year, county and region of isolation, and pulsotype.

The seven isolates depicted are described in Table 1. Isolate 2 is TEM-1 beta-lactamase positive, the remaining isolates are beta-lactamase negative.

(14/30) and IV (9/30). The Hif isolate and the blood isolate were both biotype I.

Twelve STs were represented, including ST1282 and ST1287 not previously registered in the MLST database (Table 1). Eleven of the 12 STs belonged to nine CCs; the last (ST1287) could not be assigned to a CC. ST1197 (CC395) was the most frequent ST (10/30). Two STs (ST1197 and ST836) encompassed both biotype III (ODC negative) and IV isolates (ODC positive).

ftsI/PBP3 genotypes

Ten *ftsI* alleles, encoding seven PBP3 substitution types and carried by one to 10 isolates, were observed (Table 1). Two *ftsI* alleles (*ftsI-2* and *ftsI-4*) were present in unrelated STs (Figure 1).

The majority of isolates (proportion: 23/30) had the N526K first stage substitution and were thus categorised as group III (11/30) or group III+(12/30). Seven isolates (7/30) had the R517H first stage substitution and were thus categorised as group III-like (3/30) or group III-like+(4/30).

The third stage substitution L389F was present in 16 of 30 isolates (Table 1).

Clonal groups

Five clusters comprising two to 10 isolates with identical MLST-*ftsI* allelic profiles (clonal groups, CG) were identified, accounting for 22 of 30 isolates. Eight isolates of 30 had unique MLST-*ftsI* allelic profiles, including the single Hif isolate and the single invasive isolate (Table 1).

CG2 (n=10 isolates) was found in two separate geographical regions (Oslo region and Western Norway) during a period of three years. To confirm genetic relationship, PFGE of seven isolates belonging to CG2 (one beta-lactamase positive and six beta-lactamase negative isolates) was performed, and four related pulsotypes (1–4) were observed (Figure 2). Pulsotypes 2 (n=2), 3 (n=1) and 4 (n=1) differed from pulsotype 1 (n=3) by two, two and three bands, respectively. Pulsotype 1 was observed in both geographical regions. No pulsotypes differed by more than five bands.

The three CG4 isolates were sampled during a period of four days from one hospitalised patient and two outpatients related to the same hospital. CG5 (n=4) included two patients from the same household (patients 27 and 28) and was restricted to one hospital department within a period of 16 days.

The clonal groups (and the single strains) differed considerably with respect to PBP3 resistance genotypes and co-resistance (Table 1). Notably, in contrast to CG1 and CG2, the third stage L389F substitution was present in CG3, CG4 and CG5. CG1 and CG2 had no co-resistance to non-beta-lactams, whereas CG4 was multidrug resistant.

Table 2

Antimicrobial susceptibility of *Haemophilus influenzae* isolates with high-level penicillin-binding protein 3-mediated beta-lactam resistance (high-rPBP3), Norway, 2006–2013 (n = 30)

Agents	MIC range (mg/L)	MIC50 (mg/L)	MIC90 (mg/L)	Breakpoints (S≤/R>)[a]	R or I/R n (%)[b]
Ampicillin[c]	1 –≥8	4	≥8	1/1	29 (97)
Amoxicillin[d]	4 – 32	8	8	2/2	30 (100)
Cefuroxime	2 –≥16	8	≥16	1/2	1 (3) / 29 (97)
Cefotaxime	≤0.12 –≥4	0.25	1	0.12/0.12	26 (87)
Ceftriaxone	≤0.03 – 2	0.12	0.5	0.12/0.12	14 (47)
Cefixime	0.12 –≥2	0.5	≥2	0.12/0.12	29 (97)
Cefepime	0.25 –≥4	1	2	0.25/0.25	28 (93)
Ceftaroline	0.03 – 0.25	0.12	0.25	0.03/0.03	26 (87)
Imipenem	≤0.5 – 2	1	1	2/2	0 (0)
Meropenem[e]	0.12 – 2	0.25	0.5	0.25/1[e]	9 (30) / 1 (3)
Ciprofloxacin	≤0.06 – 2	≤0.06	0.25	0.5/0.5	3 (10)
Levofloxacin	≤0.03 – 1	≤0.03	0.25	1/1	0 (0)
Chloramphenicol	0.25 –≥8	0.5	≥8	2/2	4 (13)
Rifampicin[f]	0.25 – 1	0.5	0.5	1/1[f]	0 (0)
Tetracycline	0.5 –≥8	0.5	≥8	1/2	0 (0) / 4 (13)
Doxycycline	0.25 – 2	0.25	2	1/2	4 (13) / 0 (0)
Minocycline	0.12 – 0.5	0.25	0.5	1/2	0 (0) / 0 (0)
Trimethoprim-sulfamethoxazole	0.12 –≥4	0.5	≥4	0.5/1	0 (0) / 13 (43)
Erythromycin[g]	2 – 4	2	4	0.5/16[g]	30 (100) / 0 (0)
Clarithromycin[g]	2 – 16	4	8	1/32[g]	30 (100) / 0 (0)
Azithromycin[g]	≤0.25 – 1	0.5	0.5	0.12/4[g]	30 (100) / 0 (0)
Roxithromycin[g]	2 – 16	4	8	1/16[g]	30 (100) / 0 (0)
Telithromycin[g]	0.5 – 2	1	1	0.12/8[g]	30 (100) / 0 (0)

I: intermediate; MIC: minimum inhibitory concentration; R: resistant; S: susceptible.

[a] European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [6]. The I category is inferred from the S and R breakpoints.

[b] Isolates categorised as R (when only S and R categories exist) or as I/R (when an I category is defined).

[c] Beta-lactamase positive isolates (n = 11) tested in the presence of sulbactam (2:1 ratio).

[d] Beta-lactamase positive isolates (n = 11) tested in the presence of clavulanic acid (2:1 ratio).

[e] Meningitis breakpoints. All isolates were susceptible according to general breakpoints (2/2).

[f] Prophylaxis breakpoints.

[g] Macrolide breakpoints categorise the wild type population as intermediately susceptible.

Penicillin-binding protein 3-mediated resistance

All strains were categorised as resistant to at least one of the extended-spectrum cephalosporins cefotaxime, ceftriaxone, cefixime, cefepime and ceftaroline; proportions of resistant isolates varied from 14/30 for ceftriaxone to 29/30 for the oral agent cefixime (Table 2). In general, strains with the N526K first stage substitution had higher MICs to extended-spectrum cephalosporins (except cefixime) than R517H strains. Furthermore, the third stage L389F substitution was generally associated with higher MICs to extended-spectrum cephalosporins and meropenem. Notably, eight of 12 group III+ isolates (N526K, S385T and L389F) were non-susceptible to meropenem (Table 3).

Individual susceptibility profiles and resistance genotypes are shown in Figure 3. In contrast to other group III isolates, the single invasive ST1287 isolate (isolate number 18) was categorised as susceptible to ampicillin (MIC = 1 mg/L), cefotaxime (MIC ≤ 0.12 mg/L), ceftriaxone (MIC ≤ 0.03 mg/L), cefixime (MIC = 0.12 mg/L) and cefepime (MIC = 0.25 mg/L). Conversely, the single ST124 Hif isolate (isolate number 21) generally expressed higher resistance levels to beta-lactams than other group III+ isolates.

Figure 3. Year-to-year overview of *Haemophilus influenzae* isolates with high-level penicillin-binding protein 3-mediated beta-lactam resistance (high-rPBP3), Norway, 2006–2013 (n=30)

Table 3

Penicillin-binding protein 3 (PBP3) groups and susceptibility to cephalosporins and carbapenems of *Haemophilus influenzae* isolates with high-level PBP3-mediated beta-lactam resistance (high-rPBP3), Norway, 2006–2013 (n=30)

Agents	Third stage high-rPBP3[a]				Second stage high-rPBP3[b]			
	Group III+ (n=12)		Group III-like+ (n=4)		Group III (n=11)		Group III-like (n=3)	
	MIC_{50} (mg/L)	R or I/R n (%)[c]	MIC_{50} (mg/L)	R or I/R n (%)[c]	MIC_{50} (mg/L)	R or I/R n (%)[c]	MIC_{50} (mg/L)	R or I/R n (%)[c]
Cefuroxime	8	1 (8) / 11 (92)	8	0 (0) / 4 (100)	8	0 (0) / 11 (100)	≥16	0 (0) / 3 (100)
Cefotaxime	1	12 (100)	0.5	4 (100)	0.25	9 (82)	≤0.12	1 (33)
Ceftriaxone	0.25	10 (83)	0.25	4 (100)	0.06	0 (0)	0.06	0 (0)
Cefixime	0.5	12 (100)	≥2	4 (100)	0.25	10 (91)	≥2	3 (100)
Cefepime	2	12 (100)	1	4 (100)	0.5	10 (91)	0.5	2 (67)
Ceftaroline	0.25	12 (100)	0.12	4 (100)	0.06	10 (91)	0.03	0 (0)
Imipenem	1	0 (0)	≤0.5	0 (0)	1	0 (0)	1	0 (0)
Meropenem	0.5	7 (58) / 1 (8)[d]	0.25	0 (0) / 0 (0)[d]	0.25	2 (18) / 0 (0)[d]	0.12	0 (0) / 0 (0)[d]

[a] L389F present. Group III+: S385T, L389F and N526K; group III-like+: S385T, L389F and R517H.

[b] L389F absent. Group III: S385T and N526K; group III-like: S385T and R517H.

[c] According to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [6]. The exact breakpoints are presented in Table 2. Isolates categorised as I/R (when an I category is defined) or as R (when only S and R categories exist).

[d] Meningitis breakpoints (0.25/1) used for categorisation. All isolates were susceptible according to general breakpoints (2/2).

Co-resistance

A total of 11 of 30 strains produced beta-lactamase and all were TEM-1 positive (Table 1). Co-resistance to trimethoprim-sulfamethoxazole (13/30) was frequent. Four of 30 isolates were resistant to chloramphenicol and tetracycline, including the three CG4 isolates and a single isolate belonging to the same clonal complex (CC503) as those forming the CG4. No isolates expressed increased resistance to macrolides or rifampicin (Table 3).

Five of 30 isolates had ciprofloxacin MIC >0.06 mg/L and possessed amino acid substitutions in the QRDR of *GyrA* and/or *ParC*. Three isolates, all belonging to CG4, had substitutions in both proteins and were clinically resistant to ciprofloxacin. Two isolates, both CG3, had only one significant *GyrA* substitution and quinolone MICs within the susceptible category (Table 4).

Case reports

The clinical course was evaluated for three patients with well-defined serious infections most likely caused by one of the study isolates (patients and isolates 18, 27 and 28). Isolate 18 was cultured from the blood of a patient with X-ray confirmed pneumonia, chronic obstructive pulmonary disease (COPD), hypogammaglobulinemia, chronic lymphocytic leukaemia and myasthenia gravis (Table 1). The strain was categorised as susceptible to cefotaxime (MIC ≤0.12 mg/L, Figure 3). The patient responded well to initial parenteral therapy with cefotaxime (three days) followed by ciprofloxacin orally.

Isolates 27 and 28 belonged to CG5 and were beta-lactamase positive and resistant to ampicillin-sulbactam (MIC = 8 mg/L) and cefotaxime (MIC = 1 mg/L) according to EUCAST breakpoints [6]. Both were isolated from sputum in patients with X-ray confirmed pneumonia (C-reactive protein ≥160 mg/L; norm: <5 mg/L) and significant co-morbidity. Patient 27 (cardiovascular disease) responded to therapy with cefotaxime (dosage 2 g three times a day) after initial treatment with benzylpenicillin. Patient 28 (disseminated cancer) responded to ciprofloxacin after initial treatment with piperacillin-tazobactam.

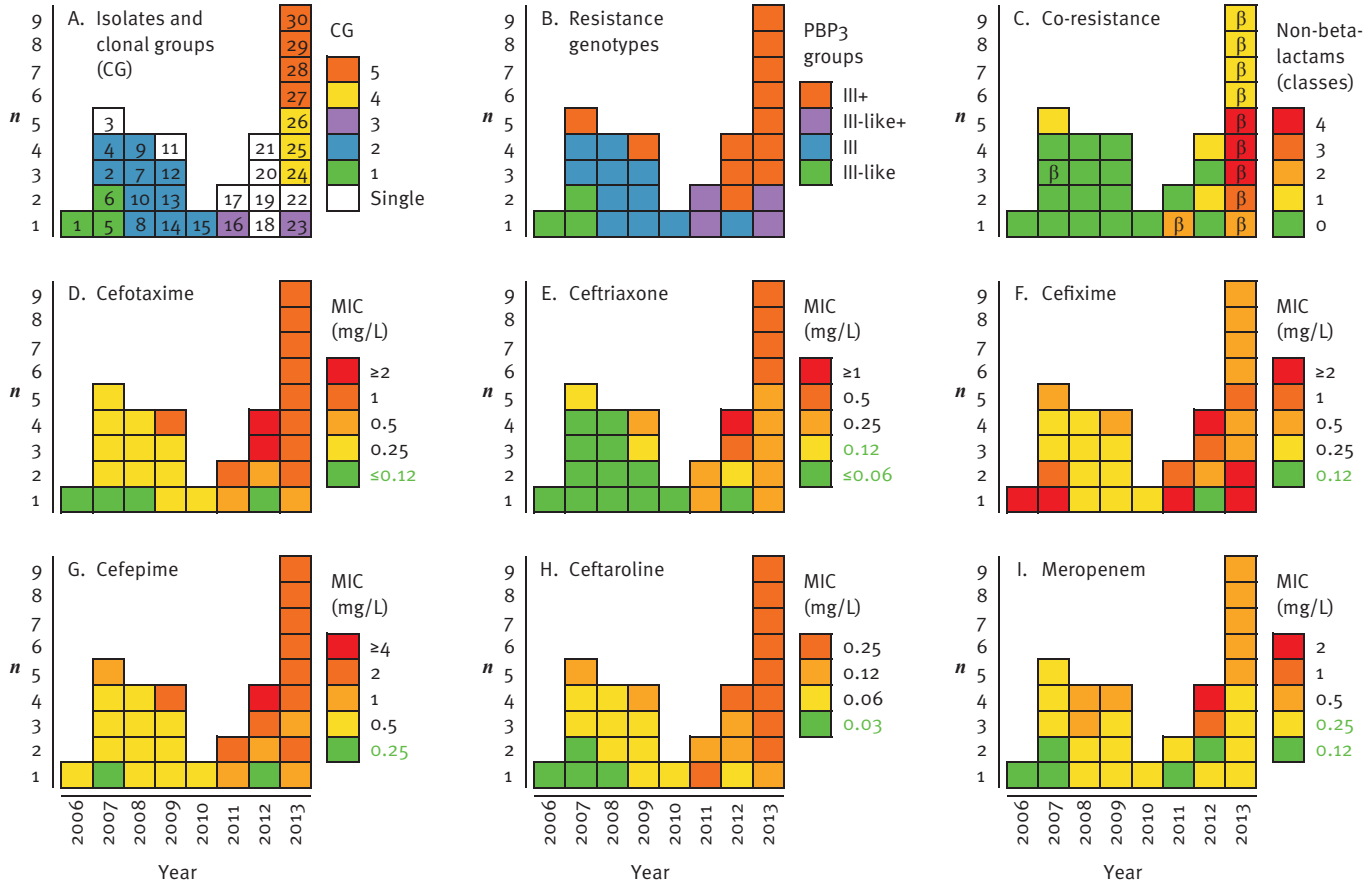
Discussion

We have characterised 30 clinical *H. influenzae* isolates from Norway with high-level PBP3-mediated beta-lactam resistance, including 23 isolates possessing both the S385T and N526K substitutions. Previously, isolates with this genotype (group III and group III+) have primarily been reported from Japan [14] and Korea [15].

The prevalence of low-rPBP3 in respiratory NTHi in Norway has increased gradually since 2001 and was estimated to 15% in 2007; 113 low-rPBP3 and three high-rPBP isolates (isolates 4–6 in this study) were reported among 795 surveillance isolates in 2007 [8]. When the prevalence of low-rPBP3 in Japan in the 1990s reached approximately the same level as in Norway in 2007, the prevalence of high-rPBP3 isolates increased from zero to 29% in few years [14]. Similar observations were reported from Korea during the 2000s [15]. NTHi was characterised as an emerging pathogen in a most

Figure 3

Year-to-year overview of *Haemophilus influenzae* isolates with high-level penicillin-binding protein 3-mediated beta-lactam resistance (high-rPBP3), Norway, 2006–2013 (n=30)



MIC: minimum inhibitory concentration; PBP3: penicillin-binding protein 3.

Colours indicate the following parameters: A, CGs (identical MLST-*fstI* profiles, Table 1); B, PBP3 resistance genotypes (grouped according to substitutions in positions 385, 389, 517 and 526, Table 1); C, co-resistance to non-beta-lactam antimicrobial agents (number of classes, including low-level resistance to quinolones) and beta-lactamase (β); D-I, MICs (broth microdilution) to extended-spectrum cephalosporins and meropenem.

Green numbers on the MIC scale indicate values below the European Committee on Antimicrobial Susceptibility Testing (EUCAST) S-breakpoint [6]. Each box indicates the same isolate in all diagrams. Isolates collected the same year (columns) are sorted by PBP3 groups, clonal groups and cefotaxime MIC.

recent review article [1]. Emphasising the emergence and spread of strains with PBP3-mediated resistance, the authors recommended implementation of standardised surveillance protocols and typing methodologies.

We recently suggested MLST-*fstI* typing as a tool for molecular surveillance of PBP3-mediated resistance in *H. influenzae* [8]. By this approach, DNA-based resistotyping using the transpeptidase region as an additional allele is combined with MLST allelic profiles. This sequence is readily available if PBP3 resistance genotyping is done by sequencing. In that study [8], MLST clonal complexes corresponded well to PFGE clusters. Addition of the *fstI* allele to MLST allelic profiles increased the discriminatory power compared with MLST alone and MLST combined with PBP3 substitution patterns, without compromising consistency with PFGE-based grouping. The observation that CG2 in the present study (and most MLST-*fstI* types in the previous study) encompassed related pulsotypes supports the validity of the typing scheme although discriminatory power is inferior to PFGE. Increasing the length of the *fstI* fragment could improve resolution but this would most likely be at the cost of reduced sensitivity for clone detection.

So far, the molecular epidemiology of high-rPBP3 strains is poorly described. However, two invasive group III-like isolates recently reported from Spain had MLST allelic profiles (ST155 and ST1118) identical or closely related to CG1 in the present study; the Spanish isolates and CG1 also had identical PBP3 substitution patterns as far as comparison is possible [3]. These observations suggest that CG1 and the two Spanish isolates may be representatives of a virulent high-rPBP3 NTHi clone distributed within Europe.

Table 4

GyrA/ParC amino acid substitutions in *Haemophilus influenzae* isolates with high-level penicillin-binding protein 3-mediated beta-lactam resistance (high-rPBP3) and raised minimum inhibitory concentrations to quinolones, Norway, 2006–2013 (n = 5)[a]

Isolate[b]	MICs (mg/L) and breakpoints (S≤/R>)[c]		GyrA[d]		ParC[d]		
	CIP (0.5/0.5)	LEV (1/1)	S84	D88	S84	N138	G206
16	0.25	0.25	L	–	–	–	R
23	0.25	0.25	L	–	–	–	R
24	2	1	L	N	I	S	–
25	2	1	L	N	I	S	–
26	2	1	L	N	I	S	–

CIP: ciprofloxacin; LEV: levofloxacin; MIC: minimum inhibitory concentration; –: no substitution.

[a] The remaining 25 isolates had ciprofloxacin MICs ≤ 0.06 mg/L and levofloxacin MICs ≤ 0.03 mg/L.
[b] Further characteristics are described in Table 1.
[c] European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [6].
[d] Amino acid substitutions in GyrA (64–177) and ParC (66–236).

Travel history suggested that one isolate in this study may have been imported from Thailand, although an isolate with identical MLST-*ftsI* profile was observed in Norway two years earlier (CG3).

In this study, CG4 and CG5 were respectively restricted to one hospital (both hospitals located in Western Norway) and sampled within short time periods. Two of the CG5 isolates were sampled from persons within the same household. Person-to-person transmission of nontypeable *H. influenzae* has been reported previously, including an outbreak with an amoxicillin-resistant strain in a respiratory ward [27] and intrafamilial transmission of rPBP3 strains [28]. These observations highlight the importance of hygiene measures in health institutions to prevent nosocomial spread.

The observation of identically mutated *ftsI* alleles in unrelated STs suggests horizontal gene transfer (HGT). Similar observations have been reported by us previously [8]. Thus, resistant strains may have developed in Norway by HGT of alleles conferring resistance; these alleles may have been imported at an earlier stage or have evolved locally. The observation of allele mosaicism in *H. influenzae* [29] supports this interpretation and strongly suggests that allele transfer has occurred via transformation and recombination with the chromosome.

By EUCAST clinical breakpoints [6], all isolates were categorised as resistant to at least one extended-spectrum cephalosporin. Clinically relevant differences in resistance profiles between PBP3 groups were observed. Isolates possessing the third stage substitution L389F were generally more resistant to cephalosporins than the remaining isolates, consistent with the results of a previous investigation using site-directed mutagenesis and transformation to study the impact of PBP3 substitutions on beta-lactam susceptibility [13]. Notably, most L389F positive isolates in the present study were ceftriaxone resistant, while all isolates lacking this substitution were ceftriaxone susceptible. The three-stage classification system used in this study, separating between isolates with and without the L389F substitution, appears to reflect the stepwise development of PBP3-mediated resistance better than systems based on S385T and R517H/N526K only.

We evaluated the effectiveness of the antimicrobial therapy for three patients with X-ray confirmed pneumonia associated with study strains. Notably, one patient responded to therapy with high dosage of cefotaxime although the isolate (from sputum) was categorised as resistant (MIC = 1 mg/L) according to EUCAST clinical breakpoints [6]. This is consistent with current EUCAST non-species related breakpoints for cefotaxime based on a dose of at least 2 g three times a day (R > 2 mg/L) [6]. The isolate would have been categorised as susceptible to cefotaxime by CLSI breakpoints for *H. influenzae* (S ≤ 2 µg/mL) [7].

The single ST1287 blood isolate from an immunocompromised patient with pneumonia is to our knowledge the first reported invasive group III high-rPBP3 isolate from Europe. This strain generally expressed lower MICs to beta-lactams than noninvasive group III study isolates and was categorised as cefotaxime susceptible (MIC ≤ 0.12 mg/L). The patient responded well to cefotaxime therapy.

Conversely, the single Hif group III+ isolate was more resistant than nontypeable isolates with comparable genotypes. These observations suggest that

strain-associated mechanisms other than PBP3 substitutions may modify resistance levels in rPBP3 isolates. Increased efflux due to mutations in the *acrR* gene, encoding a repressor of the AcrAB efflux pump, is associated with high-level macrolide resistance in *H. influenzae* and may increase resistance to ampicillin in rPBP3 strains [30]. The *acrR* gene was not sequenced in the present study but all isolates expressed wild-type MICs to macrolides.

Co-resistance was more frequent than expected. The proportions of trimethoprim-sulfamethoxazole resistant and beta-lactamase positive isolates were twice and three times, respectively, the overall reported prevalences in respiratory *H. influenzae* isolates (n = 677) in a Norwegian nationwide surveillance study in 2011 [31]. Similarly, resistance rates for ciprofloxacin, tetracycline and chloramphenicol in this study were 10% (3/30), 13% (4/30) and 13% (4/30), respectively, compared with 0.1%, 0.4% and 0.7% in the 2011 surveillance report.

EUCAST define resistance to ciprofloxacin as an exceptional phenotype in *H. influenzae* and recommend that such isolates are referred to a reference laboratory [5,6]. Quinolone resistance in this species is usually due to QRDR substitutions in subunit A of topoisomerase II (*GyrA*) and subunit A of topoisomerase IV (*ParC*). Resistance levels depend largely on the number of substitutions in the positions 84 and 88 in both proteins [32]. Isolates with single substitutions are usually low-level resistant but other mechanisms such as the transferable plasmid-mediated acetyl transferase gene *aac(6')-Ib-cr* may increase resistance [33,34]. The QRDR substitution patterns and quinolone MICs observed in this study are consistent with previous reports [26,32]. The nalidixic acid 30 µg disk is superior to the ciprofloxacin 5 µg disk for detection of isolates with low-level quinolone resistance and may be used for screening [6,26].

Quinolone resistance in *H. influenzae* is associated with hypermutability [33]. It seems likely that hypermutability also increases the ability to acquire PBP3 substitutions by spontaneous mutations, favouring the development of strains with both resistance mechanisms. To our knowledge, CG4 represent the first ciprofloxacin-resistant high-rPBP3 isolates reported outside Japan, and the first clonal cluster ever with this particular combination of resistance mechanisms.

The CG4 isolates had TEM-1 beta-lactamase and were resistant to chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole. This resistance profile suggests the presence of a conjugative large plasmid [35], now recognised as an integrating and conjugative element (ICE) designated ICE*Hin1056* [36]. The resistotype of CG4 might thus be the result of two independent factors: hypermutability, leading to PBP3 and QRDR substitutions, and the acquisition of ICE*Hin1056*. Hypermutable *H. influenzae* are prevalent in cystic fibrosis (CF) patients [37]. One CG4 isolate was found in sputum from a CF patient; another isolate was from nasopharynx of a patient with primary ciliary dyskinesia (PCD). High antibiotic pressure in these patient categories may contribute to selection of multidrug resistant strains.

The ST of CG4 (ST159) is associated with increased virulence in chronic obstructive pulmonary disease due to an additional IgA1 protease, encoded by the *igaB* gene [38].

The emergence of clonally related high-rPBP3 isolates in Norway is a cause for concern. As previously reported from Japan and Korea, a shift from low-level to high-level rPBP3 might alter the epidemiological situation dramatically in few years [14,15]. A similar development in Europe would threaten current recommendations for empiric antimicrobial treatment in severe disease [1]. The situation urges for improved surveillance systems for antimicrobial resistance in *H. influenzae* in Europe.

Acknowledgements

This work was supported by a grant from Vestfold Hospital Trust. We thank the following for contributing with bacterial isolates and/or clinical data: Haima Mylvaganam (Department of Microbiology, Haukeland University Hospital); Reidar Hjetland and Ingerid Skarstein (Department of Microbiology, Førde Hospital); Truls Leegaard (Department of Microbiology, Akershus University Hospital); Sølvi Noraas and Irene Beate Olsøy (Department of Medical Microbiology, Hospital of Southern Norway Trust); and Pål Arne Jenum (Department of Medical Microbiology, Vestre Viken Hospital Trust). We thank Astrid Lia and Anja Hannisdal (Vestfold Hospital Trust) for excellent technical assistance; Dominique A. Caugant, Elisabeth Fritzsønn and Anne Witsø (Norwegian Institute of Public Health) for performing MLST and capsular serotyping; Andrew Jenkins (Telemark University College, Bø) for help with analysis of PFGE band patterns; Haima Mylvaganam and Dominique A. Caugant for valuable comments to the manuscript; and the Norwegian Surveillance Programme for Antimicrobial Resistance (NORM) for access to the surveillance database. We also acknowledge use of the *Haemophilus influenzae* MLST website.

Conflict of interest

None declared.

Authors' contributions

DS conceived and coordinated the study; AS and BEK contributed to study design. DS, OBN and MS collected bacterial isolates and clinical information; DS performed or was responsible for testing and characterisation of isolates in collaboration with GK and EM (MIC determination); MS (MLST and capsular serotyping) and ILA (remaining molecular analyses). DS analysed and interpreted data and wrote the first draft. All authors participated in interpretation of results, critically revised the draft for intellectual content and approved the final article.

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Measles outbreak in Greater Manchester, England, October 2012 to September 2013: epidemiology and control

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Citation style for this article:
Pegorie M, Shankar K, Welfare WS, Wilson RW, Khiroya C, Munslow G, Fiefield D, Bothra V, McCann R. Measles outbreak in Greater Manchester, England, October 2012 to September 2013: epidemiology and control. Euro Surveill. 2014;19(49):pii=20982. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20982

Article submitted on 18 March 2014 / published on 11 December 2014

This paper describes the epidemiology and management of a prolonged outbreak of measles across the 2.7 million conurbation of Greater Manchester in the United Kingdom. Over a period of one year (from October 2012 to September 2013), over a thousand suspected measles cases (n=1,073) were notified across Greater Manchester; of these, 395 (37%) were laboratory-confirmed, 91 (8%) were classed as probable, 312 (29%) were classed as possible and 275 (26%) excluded. Most confirmed and probable cases occurred in children within two age groups – infants (too young to be eligible for measles-mumps-rubella (MMR) vaccination according to the national immunisation programme) and children aged 10–19 years (low vaccine uptake in this cohort because of unfounded alleged links between the MMR vaccine and autism). During this one year period, there were a series of local outbreaks and many of these occurred within the secondary school setting. A series of public health measures were taken to control this prolonged outbreak: setting up incident management teams to control local outbreaks, a concerted immunisation catch-up campaign (initially local then national) to reduce the pool of children partially or totally unprotected against measles, and the exclusion of close contacts from nurseries and school settings for a period of 10 days following the last exposure to a case of measles.

Background

The number of notified measles cases in Europe fell from 28,203 to 7,499 between 2003 and 2009 [1]. Since then, there have been a number of measles outbreaks especially in central and western Europe, with a peak of cases reported in 2011 (32,124 cases reported) [1,2]. France was the most affected country, with 47% of cases in Europe in 2011, while several other countries have also reported a considerable number of cases including Bulgaria, Germany, Italy, Romania, Spain, Ukraine, and the United Kingdom (UK) [1,2].

In the UK, a measles-containing vaccine was introduced into the national childhood immunisation schedule in 1968. Since 1988, the combined measles-mumps-rubella (MMR) vaccine has been used [3]. In the early 1990s, the number of confirmed cases of measles fell significantly from ca 80,000 cases in 1988 to ca 100 cases in 2000, as uptake of MMR vaccination increased, to the point where sustained transmission was interrupted [4]. Unfounded fears about a potential link between MMR vaccine and autism [5,6] in the late 1990s damaged public confidence in MMR vaccine, reduced uptake, and increased the risk of sustained transmission [7,8] leading to the number of confirmed measles cases in England and Wales increasing to above 1,000 in 2008 and remaining above 1,000 since, except for the year 2010 when 380 cases were confirmed. In the UK, recent outbreaks have mainly occurred in areas or specific groups with known low coverage of MMR vaccination [9].

Greater Manchester is a city region in the north-west of England with a population of around 2.7 million, administratively divided into 10 local authorities with different population sizes and characteristics. Recent MMR vaccine uptake has been high, with 93.3% of children having received their first dose of MMR vaccine by their second birthday in 2011–2012 (national mean: 91.2%) and 88.1% of children having received two doses of MMR vaccine by their fifth birthday in 2011–2012 (national mean: 86%) [10].

However, there was an under-vaccinated cohort aged between 10 and 16 years across Greater Manchester, estimated at 31,600 children in April 2013. Of these, 11,993 were totally unvaccinated, and 19,644 had

Table

Number of notifications of suspected and confirmed cases of measles, Greater Manchester, England, 2007–2013

Year of receipt of notifications	Total number of notifications received	Number of laboratory-confirmed measles cases among the notifications received
2007	212	22
2008	235	26
2009	394	40
2010	310	30
2011	246	21
2012	664	149
2013	894	229

received only one dose of MMR vaccine [11]. Of those aged 10–16 years in Greater Manchester, 10.4% were thought to be susceptible to measles [12]. This cohort has been the target of previous local catch-up vaccination campaigns with varying success [12] and was being targeted by a national catch-up vaccination campaign (2013–2014) [13].

Surveillance system in England

All doctors in England and Wales must notify the 'Proper Officer' of the relevant local authority (usually staff of the local Public Health England Centre) of clinically-suspected cases of measles. Other clinicians are encouraged to do the same.

The local Public Health England (PHE) team will record and undertake local follow-up including assessing how likely this is to be measles. The local team will send an oral fluid swab kit to the suspected case. This kit is designed for use by the patient and is posted directly to the Virus Reference Department, PHE Microbiology Services Colindale, London (the national reference laboratory) for IgM / IgG and RNA testing.
Test results are sent to both the relevant clinician and PHE. All laboratories in England notify PHE of confirmed measles cases. PHE receives and collates reported cases with a negative result and positive cases and reports confirmed cases to the European surveillance network (EUVAC-NET) on a monthly basis. This is described in more detail elsewhere [14].

In this report, we describe a prolonged outbreak of measles in Greater Manchester, the control measures that were taken and the lessons learned.

Methods

This report includes all reported cases of measles in residents of Greater Manchester between 1 October 2012 and 30 September 2013.

Case definitions

Case definitions were primarily devised for case management purposes and were adapted from national guidelines [3] to fit the local epidemiology of the outbreak. The national case management guidelines separate likely (probable) cases and unlikely (possible) cases. These are consistent with those used in a recently published outbreak report [15].

Figure 1

Number of confirmed, probable and possible cases of measles by week of rash onset, Greater Manchester, England, October 2012–September 2013 (n=798)

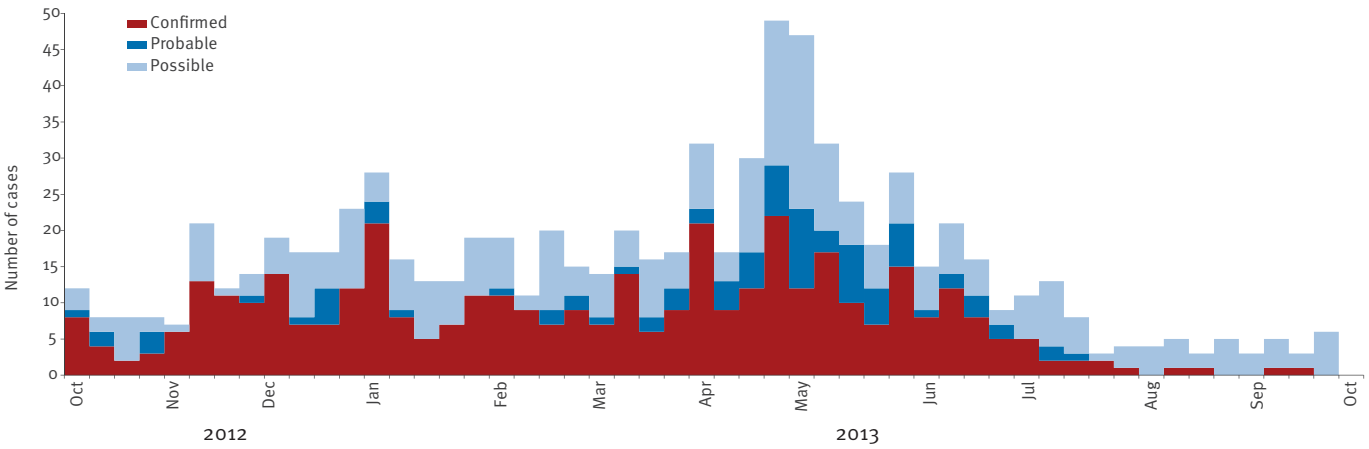
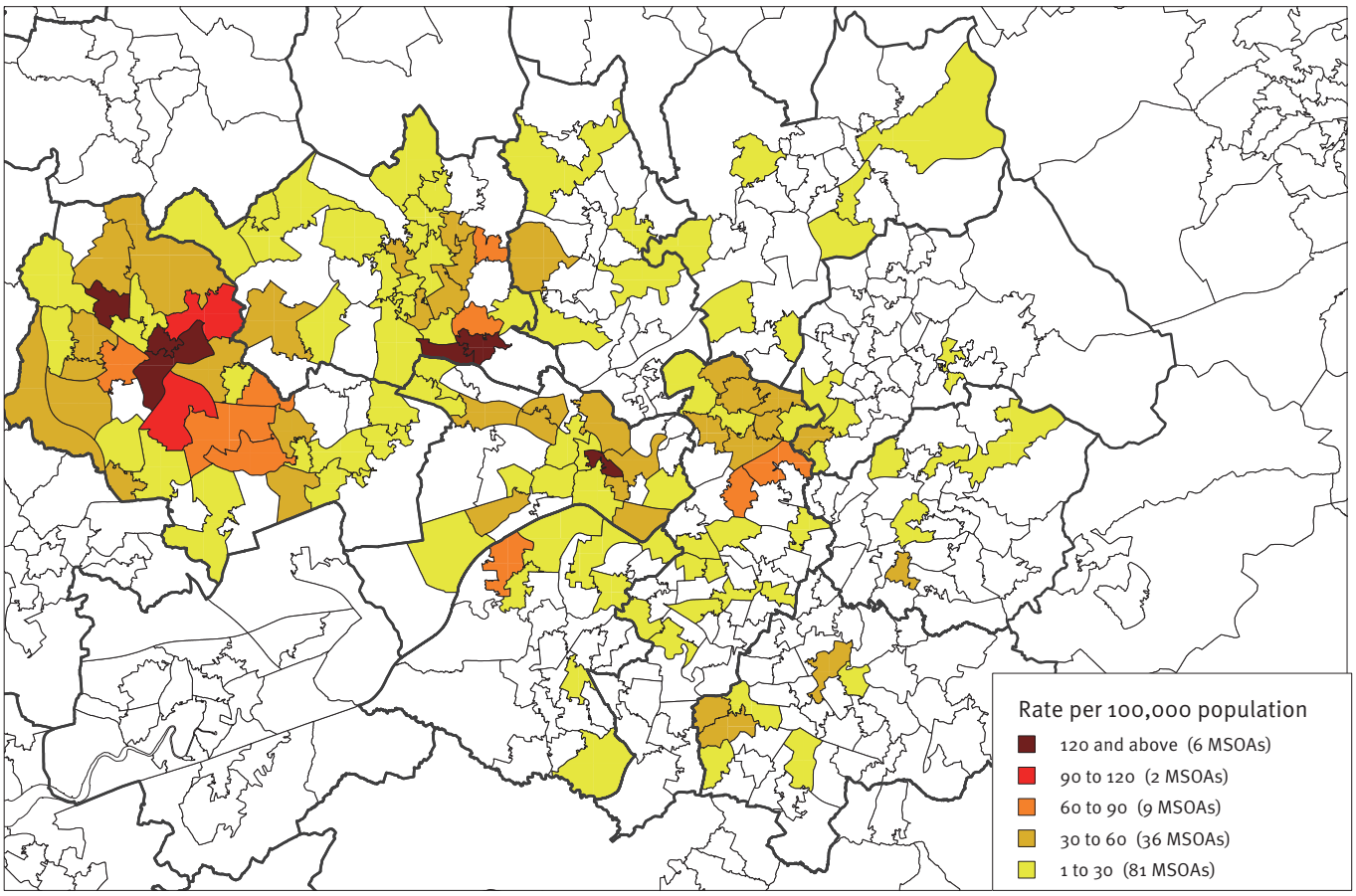


Figure 2

Measles rates by Middle Super Output Area (MSOAs)[a], Greater Manchester, England, October 2012–September 2013 (n=486 probable and confirmed cases)



[a] Super Output Areas (SOAs) are small areas of consistent size across the country used by the United Kingdom Office for National Statistics and are not subjected to regular boundary change. Each Middle Super Output Area (MSOA) has a population of 5,000–15,000 people and contains 2,000–6,000 households.

Confirmed case: measles IgM-positive result in blood or oral fluid in the absence of a history of recent vaccination or confirmed wild measles RNA positive in any clinical specimen.

Probable case: case notified by a clinician as suspected measles who was not fully vaccinated with two doses of MMR vaccine and met one or more of the following criteria: (i) epidemiological link (recent contact with a laboratory-confirmed case of measles); (ii) member of the travelling community or orthodox Jewish community; (iii) link to a setting with a known outbreak; or (iv) aged over 16. Such cases are classified as likely as per national guidelines. If a laboratory result was received for a probable case, they were reclassified as confirmed or discarded depending on the result.

Possible case: case notified by a clinician as suspected measles but not meeting the above criteria (classified as unlikely as per national guidelines [1]). If a laboratory result was received for a possible case, they were reclassified as confirmed or discarded depending on the result.

Discarded case: a previously notified case with negative test result at the national reference laboratory in an adequate and appropriately timed specimen of oral fluid or blood.

Cases notified who were from the orthodox Jewish community and the travelling community were considered to be more likely to be actual measles based on known low vaccine coverage in these groups, increased risk of introduction of measles and recent local epidemiology. Confirmed, probable and where appropriate, possible cases were included in this analysis whereas discarded cases were excluded. To ensure that this report describes a single outbreak, cases (and associated contacts) with confirmed genotype other than D8 were excluded. Only one case with a genotype other than D8 was identified during the period reported here: a case with genotype B3.

Reporting clinicians were asked to submit samples for local PCR by the PHE laboratory in Manchester, where possible. A salivary testing kit was posted to all reported cases for self-administration and direct return to the national reference laboratory.

The likely site of measles transmission or acquisition associated with each case was recorded. If two or more cases were linked to a particular setting, further investigation was undertaken, including social network analysis. Social network diagrams were drawn using Microsoft Visio.

Strategic coordination

Outbreak control teams (OCT) were established for affected individual local authorities, with oversight by a Measles Strategy Group. The strategy group reviewed case definitions based on the evolving epidemiology of the outbreak and agreed on the strategy for optimising efficiency of the overall outbreak response. In specific geographical areas, for specific periods of time where community transmission of measles was considered endemic, MMR vaccination was prioritised over the public health management of cases and contacts.

Proactive and reactive communication strategies were agreed by the strategy group. Examples include proactive engagement with local media through press releases and interviews, and regular updates which were communicated to local authorities affected by the outbreak and to clinical networks throughout Greater Manchester.

Results

Outbreak description

Between 1 October 2012 and 30 September 2013, 1,073 suspected cases of measles in Greater Manchester residents were reported by clinicians and laboratories (Figure 1). This was significantly higher than expected (Table). Of these cases, 670 were tested. Of all suspected cases reported by clinicians and laboratories, 395 (37%) were confirmed, 91 (8%) were classed as probable, 312 (29%) were classed as possible and 275 (26%) were discarded after laboratory results were known.

The highest number of reports in one week was in April 2013 (Figure 1). The outbreak mainly affected four of ten Greater Manchester local authority areas: Bolton and Wigan initially, then Salford, and lastly, Manchester (Figure 2). In 2013, there was a sharp decrease in the number of cases notified, following the start of the summer school holidays (from the end of July until early September) (Figure 1).

Two peaks were seen in the age distribution (Figure 3): those aged under two years and those aged 10–14 years. If possible cases were included (data not shown) the under two-year-olds peak would be more pronounced, probably as younger children are more

Figure 3

Measles-mumps-rubella vaccination status by age of confirmed and probable measles cases, Greater Manchester, England, October 2012–September 2013

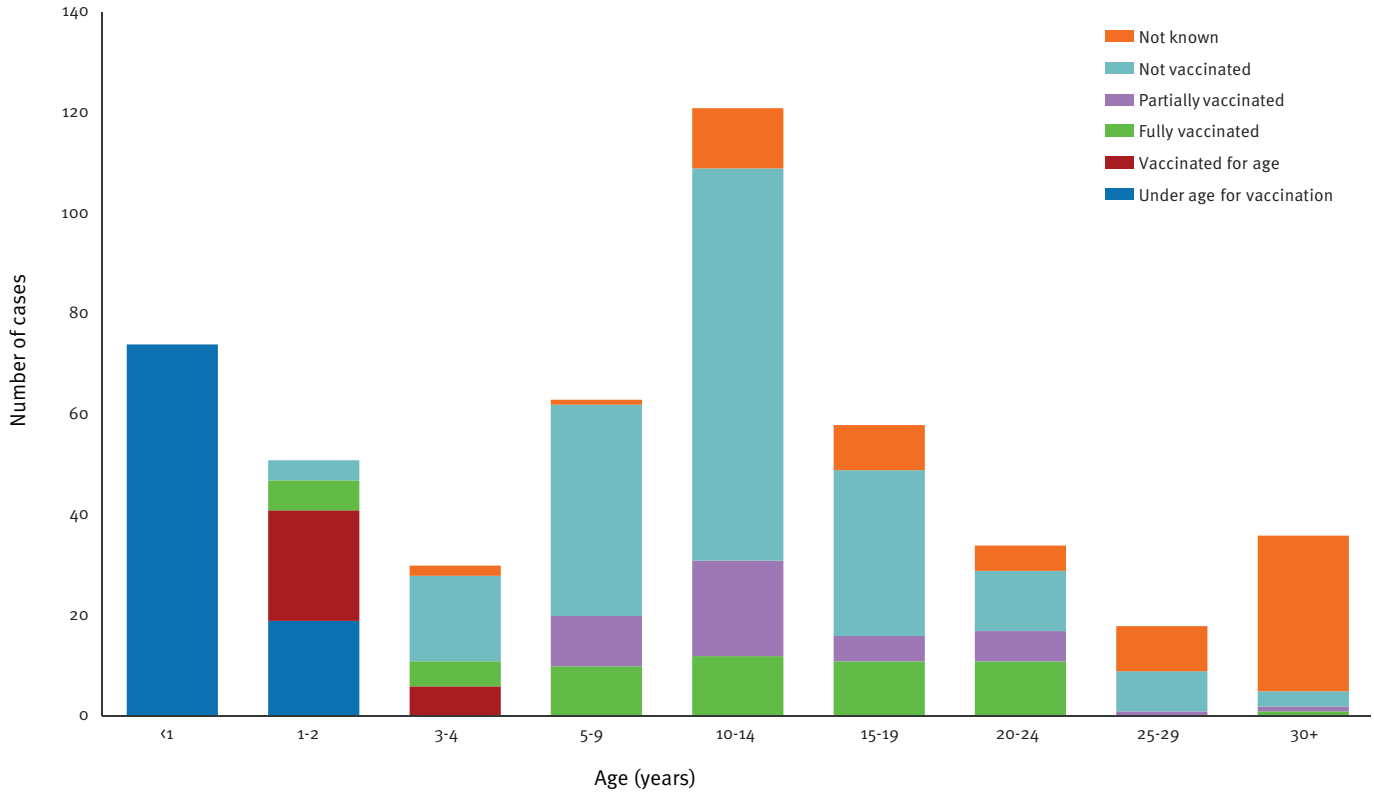
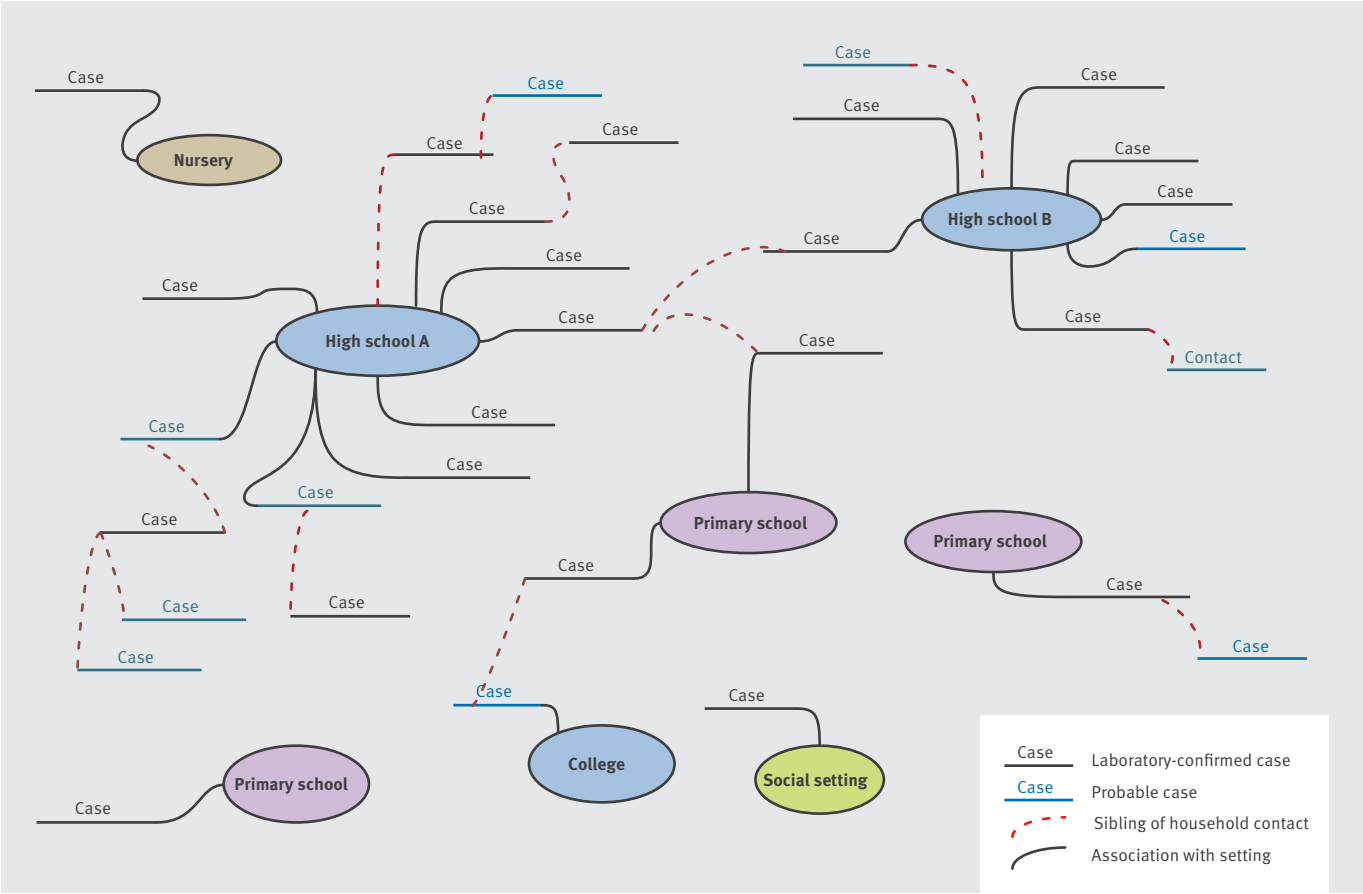


Figure 4

Example of a social network diagram representing transmission events in a high school and in household settings



likely to present to health services with rash illnesses. Information on age was available for all possible, probable and confirmed cases. The median age among confirmed cases alone was 21 years (range 0–52 years), and among confirmed and probable cases, 23 years (range 0 to 52 years). Among probable and confirmed cases 54% (264) were male. The majority of probable and confirmed cases were in those who had not had two doses of MMR vaccine. Of 486 probable and confirmed cases, only 56 cases (12%) were fully vaccinated with two doses of MMR vaccine (Figure 3).

Among the 486 confirmed and probable cases, 69 (14%) cases did not know if they were vaccinated ('Not known' in Figure 3). Of these, almost 50% were above 30 years of age.

Schools and social networks

More than 40 educational settings were involved in this outbreak, mainly secondary schools. Eight schools had more than five cases each. The two schools with the highest number of cases (ca 20) were both large secondary schools (over 1,000 pupils) with similar numbers of children who had received one dose or no dose of MMR vaccine in each school.

Due to the high proportion of susceptible 10–19 year-olds in secondary schools and the high levels of social interaction within secondary schools, a number of secondary schools were identified as probable sites of transmission. On average, within the same school, 56% (95% confidence interval (CI): 44–67) of secondary cases were in the same year group as the index case, and this proportion increased with index case age, suggesting that compared with younger school year groups, in the older school year groups, transmission events were more likely to occur between children from the same school year group (unpublished data).

Members of the Measles Strategy Group reviewed periodically the confirmed and probable cases along with any associated educational or community settings, and produced the network diagram. This network diagram was found useful to the team handling the outbreak to get a quick view of the spread of the disease.

Laboratory results

In total, 670 of the cases linked to this outbreak were tested. Genotype D8 was the predominant genotype identified in cases.

Data on hospitalisations due to secondary complications were not of adequate quality to enable analysis.

Control measures

It was not feasible or possible to formally evaluate the local control measures implemented due to resource limitations and associated opportunity cost to the health protection team, and the challenges of finding an appropriate design for evaluation which would have allowed us to measure any impact and attribute this to the intervention. Where possible, we attempted to measure impact of intervention using crude measures such as comparison of the number of pre- and post-intervention transmission events, but it was judged that this method was too crude to obtain valid and publishable results.

Management of cases

Notified cases were managed by the Greater Manchester Health Protection Team according to national guidelines [16]: where possible [3], confirmed or probable cases of measles were: (i) isolated (while in a healthcare setting), (ii) advised to avoid contact with vulnerable people, and (iii) excluded from work or educational activities, for the duration of their infectious period. Particular attention was given to cases (or susceptible contacts) who were healthcare workers. For them, a longer exclusion period of 21 days was advised, in line with UK guidelines [3].

For susceptible household contacts of a probable or confirmed case (i.e. contacts with no history of either natural measles infection or having received a measles containing vaccine), exclusion from educational settings (child-minder, nursery, primary school, secondary school, college or university) was recommended for 10 days following onset of rash in the index case. This was based on the assumption that transmission from index case to contact in this continuous exposure scenario would occur four days before appearance of the rash in the index case (the start of the infectious period for measles) and on the standard 14-day average incubation period for measles defined in national guidelines.

Awareness raising in educational and healthcare settings

Awareness of measles and the importance of exclusion from educational settings was increased among teaching staff, students and parents by sending 'warn and inform' letters via school management teams. These letters outlined the symptoms of measles, the need to seek medical assessment while being aware of the potential for transmission, measures to avoid onward transmission (including exclusion and voluntary isolation), and the importance of vaccination in primary prevention of measles.

Raising awareness among clinicians of the infectivity of measles and the importance of reducing risk of transmission to other patients by isolating suspected cases in healthcare settings was achieved proactively by sending a measles bulletin to clinicians, hospitals and local authorities, and reactively by notifying the relevant infection control teams about cases in their institution.

When a cluster was identified, a local outbreak control team was formed to manage it. Within the Health Protection Team, a measles strategy group with a nominated lead maintained an overview. Surveillance data were reviewed at least weekly.

Reducing the pool of susceptible individuals

Local measures

Both proactive and reactive approaches were used to reduce the pool of susceptible individuals. Where probable or confirmed cases were notified, a complete course of MMR vaccine was advised for all under-immunised household contacts.

Educational settings attended by confirmed or probable cases were targeted. The approach used to increase MMR vaccine uptake varied from sending letters to parents encouraging them to take any under-vaccinated children to their GP for MMR vaccination, to specific school-based immunisation sessions. In school-based immunisations sessions, priority was given to immunising children who had received no MMR dose over those who were partially immunised.

Local OCTs decided whether to offer MMR vaccine to children attending affected schools, via GPs or a school-based campaign depending on the level of susceptibility within the setting, resources available to local public health teams, and on whether a previous school-based immunisation session had already taken place in the setting or not.

In some areas, MMR vaccine was proactively offered in schools with a high number of susceptible children, especially where these schools had links to affected schools [17]. The risk of transmission between schools was highlighted early on by social network analysis (Figure 4). Early awareness and understanding of this potential for transmission allowed local teams to target 'feeder' primary schools (primary schools most likely to host younger siblings of cases in the secondary school) as sites for intervention.

Proactive approaches included: supporting local public health teams in areas most affected by the outbreak to raise awareness of the measles outbreak, promoting MMR vaccination, and raising awareness of the importance of immunity to measles among all clinical staff via a regular measles bulletin.

National campaign

In response to multiple outbreaks in different areas of England and a rise in numbers of cases nationally in 2012 and 2013, a national MMR catch-up vaccination campaign [13] was launched in April 2013, with the aim of achieving a 95% uptake of one dose of MMR vaccine in 10–16 year-olds by September 2013.

Nationally-set indicators were used to measure uptake, which included number of invitations sent and number of MMR vaccine doses ordered by primary care.

The national MMR catch-up vaccination campaign was implemented in two phases.

In Greater Manchester, in phase 1, primary care services were asked to actively identify under-immunised 10–16 year-olds using a variety of methods, including checking their own practices' electronic records [18], and to invite them for MMR vaccination using a method of their choice (usually a letter) and immunise them. GPs were remunerated for this service. Under-immunised 10–16 year-old children were defined as 10–16 year-old children who had received one dose or no dose of MMR vaccine. Priority was given to those who had received no dose of MMR vaccine.

In phase 2, the focus in Greater Manchester was on schools with low uptake. School Health Services review immunisation status when the children enter primary or secondary school and when delivering other vaccinations such as human papillomavirus (HPV) vaccine, and at school leaver's booster. Any 10–16 year-olds under-immunised for measles identified in this way were offered a dose of MMR vaccine.

The local impact of the national MMR catch-up vaccination campaign

A total of 496 (98%) of General Practices in Greater Manchester participated in the catch-up vaccination campaign. Between May 2013 and October 2013, over 10,000 invitation letters were sent out and 947 doses of MMR vaccine were given by Greater Manchester primary care services taking part in the catch-up campaign. Phase 1 of the catch-up campaign ended in September 2013, and no further letters were sent out beyond this point. Further eligible children will have been identified and immunised during phase 2 of the campaign by school nurses. No further information on numbers of 10–16 year-olds identified or numbers of 10–16 year-olds immunised is currently available.

An interim analysis of the impact of the catch-up campaign nationally has been recently published [19], which suggested achievement of the target of 95% of 10–16 year-olds having had at least one dose of MMR vaccine. Indirect impacts such as raising awareness of the risks of measles and the benefits of MMR vaccination are likely to have had a positive impact.

Discussion and conclusions

The descriptive epidemiology of this outbreak was consistent with multiple introductions of measles into partially susceptible communities resulting in a series of discrete outbreaks rather than Greater Manchester wide transmission. Some local authorities had localised outbreaks, often amplified and maintained in educational settings, in particular secondary schools, reflecting known cohorts of low coverage. This was demonstrated by the social network diagrams and the age groups affected. The outbreak control team found social network analysis useful in the early phases of localised outbreaks associated with a particular setting to understand transmission patterns and enable prioritisation of control measures. Other local authorities had only sporadic cases with no known link between them. This observed pattern may be partly the result of artefact, with contributing factors such as under-reporting of cases to the health protection team, and partly a reflection of different transmission patterns in different areas, with possible contributing factors including: different MMR vaccine uptake rates, difference in potential for onward transmission (for example, cases who do not attend educational settings), differences in social networks and socialising behaviours. This is in contrast to an earlier large outbreak in Merseyside (40 miles away from Greater Manchester), where the key transmission events were in healthcare settings [20].

Patterns of transmission differed between local authority areas. Two neighbouring areas with similar current MMR vaccine uptake levels experienced different transmission patterns: one had a localised secondary school-focused outbreak which was rapidly controlled with a proactive approach to increasing MMR vaccine uptake in the susceptible population; the other had a more widespread and longer outbreak associated with a variety of educational settings and family clusters, managed reactively. This may be due to the educational systems within the areas (including catchment areas of the schools) or due to the outbreak management strategy.

Northern areas of Greater Manchester reported more cases and outbreaks than southern areas. Although current uptake rates for MMR vaccine are high in many northern areas of Greater Manchester, historical uptake rates were not, and the estimated size of the susceptible population was higher in these areas. Absolute numbers of susceptible individuals rather than current vaccine uptake rates should be taken into account when prioritising areas for proactive control measures.

In this outbreak, the genotype confirmed was D8, a different genotype to the recent large outbreak in the neighbouring area of Merseyside [15], and the same genotype but different strain to the recent large outbreak in South Wales [20]. D8 strain has been identified in other areas: an outbreak in North Wales, cases in Cheshire and a concurrent outbreak in the traveller community [16]. The different genotyping highlighted the limited transmission risks and social networks between the two neighbouring cities.

Investigation of social settings was useful to track transmission and identify targets for intervention particularly in the early phases of the outbreak. The pictorial representation of the contextual settings along with its association with the case provided an

understanding of the progression of the spread of the disease and helped plan preventive actions.

The sharp decrease in notification of cases in the summer holidays (from the end of July to early September) supports the hypothesis that schools were the sites of transmission during this outbreak.

The public health actions of note included: reducing likelihood of onward transmission by excluding susceptible household contacts of cases from educational settings and reducing the size of the susceptible population by proactive MMR immunisation targeting high-risk educational settings.

Increasing MMR vaccine uptake in target groups via GPs was carried out as part of different aspects of the outbreak response: as part of local outbreak management, on instruction from the local outbreak control team; and as part of the national MMR vaccination catch-up campaign; this was directed nationally and GPs only commenced systematic activity once the campaign was launched in late April 2013.

Exclusion of unimmunised household contacts from educational settings was voluntary and informal. In the majority of cases, parents and schools agreed to this request but compliance with exclusion was not monitored.

The importance of isolation of infectious individuals in controlling the spread of measles has been reported on previously [21], and anecdotal evidence from this outbreak suggests that excluding infectious cases and their susceptible household contacts from educational settings was one of the most effective measures in controlling local outbreaks.

In the response to some local outbreaks, high-risk schools were identified usually based on social/educational networks and levels of MMR vaccine uptake. These schools were then targeted proactively. This seemed to be associated with rapid control in some areas. Uptake in this scenario may be better than proactive campaigns in the absence of an outbreak.

This outbreak was not associated with specific hard-to-reach groups [22] but was in the wider population. Such outbreaks highlight the problem of cohorts of under-vaccinated children. If such outbreaks continue to occur, Europe will not achieve its goal of measles elimination by 2015 [23].

This paper describes the epidemiology and management of a prolonged outbreak of measles. Data were collected for case management purposes. Standard operating procedures evolved during the outbreak. Evaluation of individual control measures was not planned before the outbreak with resultant limitations. Children interact both inside and outside of school: it was impossible to separate secondary schools and the associated out-of-school social networks as sites of transmission.

We have described a measles outbreak that predominantly affected an under-immunised cohort of teenagers and young adults, with key transmission and amplification events associated with secondary schools. Of key importance for public health practice were the thorough investigation of outbreaks, developing understanding of local transmission supported by the use of social network analysis, and the multifacetted approach to control measures, with a bundle of public health measures (both reactive and proactive) focussing on the two key elements of improving MMR vaccine uptake in the susceptible population and excluding infectious cases from settings with a high potential for onwards transmission (such as secondary schools).

Acknowledgements

We gratefully acknowledge all the NHS, PHE (formerly HPA), schools and local authority staff who contributed to outbreak management.

Conflict of interest

None declared.

Authors' contributions

MP RM DF WW VB KS CK and GM were involved in the management of the outbreak. WW and KS undertook the main data analysis. RWCW analysed the schools data. The overall paper outline was developed by WW RM and MP. All authors drafted sections of the paper. All authors commented on drafts and approved the final version.

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Clinical severity of human infections with avian influenza A(H7N9) virus, China, 2013/14

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Citation style for this article:
Feng L, Wu JT, Liu X, Yang P, Tsang TK, Jiang H, Wu P, Yang J, Fang VJ, Qin Y, Lau EH, Li M, Zheng J, Peng Z, Xie Y, Wang Q, Li Z, Leung GM, Gao GF, Yu H, Cowling BJ. Clinical severity of human infections with avian influenza A(H7N9) virus, China, 2013/14. Euro Surveill. 2014;19(49):pii=20984. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20984

Article submitted on 18 June 2014 / published on 11 December 2014

Assessing the severity of emerging infections is challenging because of potential biases in case ascertainment. The first human case of infection with influenza A(H7N9) virus was identified in China in March 2013; since then, the virus has caused two epidemic waves in the country. There were 134 laboratory-confirmed cases detected in the first epidemic wave from January to September 2013. In the second epidemic wave of human infections with avian influenza A(H7N9) virus in China from October 2013 to October 2014, we estimated that the risk of death among hospitalised cases of infection with influenza A(H7N9) virus was 48% (95% credibility interval: 42–54%), slightly higher than the corresponding risk in the first wave. Age-specific risks of death among hospitalised cases were also significantly higher in the second wave. Using data on symptomatic cases identified through national sentinel influenza-like illness surveillance, we estimated that the risk of death among symptomatic cases of infection with influenza A(H7N9) virus was 0.10% (95% credibility interval: 0.029–3.6%), which was similar to previous estimates for the first epidemic wave of human infections with influenza A(H7N9) virus in 2013. An increase in the risk of death among hospitalised cases in the second wave could be real because of changes in the virus, because of seasonal changes in host susceptibility to severe infection, or because of variation in treatment practices between hospitals, while the increase could be artefactual because of changes in ascertainment of cases in different areas at different times.

Introduction

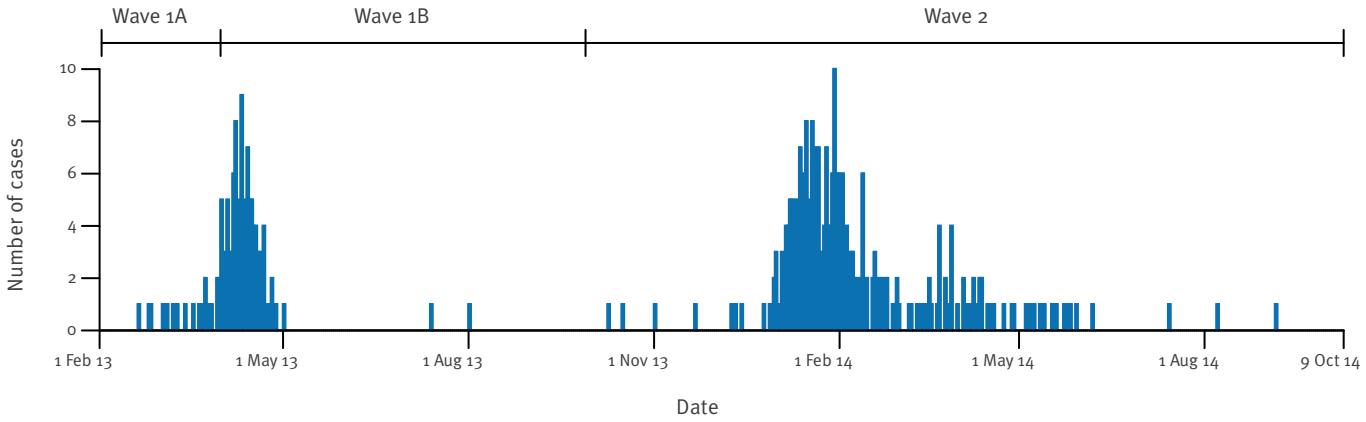
Since the first human case of infection with novel avian influenza A(H7N9) virus was identified in China in March 2013, there have been two major epidemic waves of human infections to date. The first epidemic wave, in the spring of 2013, waned during the late spring and summer [1-3], while a second major epidemic wave occurred during the winter of 2013/14 and had waned by the end of the spring of 2014 while sporadic cases have continued to be reported (as of 9 October 2014). A small number of clusters of laboratory-confirmed cases have been identified in both epidemic waves, but the virus has not appeared to have the capacity for sustained human-to-human transmission [1].

Confirmed cases of infection with influenza A(H7N9) virus have generally been identified in hospitalised patients with pneumonia [4], however, a small number of confirmed cases was identified through routine sentinel influenza-like illness (ILI) surveillance which indicates the possibility for a larger number of mild influenza A(H7N9) virus infections [5,6]. This has implications for determination of the clinical severity of influenza A(H7N9) virus infections, because the confirmed cases may not fully reflect the clinical spectrum of infections, and consequently changes in case ascertainment could lead to artefactual variation in risk of severe outcomes.

In previous work, we demonstrated that the case fatality risk among confirmed cases of infection with the 2009 pandemic influenza A(H1N1) virus was very heterogeneous and difficult to interpret [7], and we characterised the severity of influenza A(H7N9) virus infections via the risk of fatalities among hospitalised cases (the 'hospitalisation fatality risk', HFR) and the risk of fatalities among symptomatic cases (the 'symptomatic case fatality risk', CFR) [3]. In the first epidemic wave of influenza A(H7N9) virus infections in spring 2013, we estimated the HFR at 36%, and the CFR at

Figure 1

Incidence of laboratory-confirmed human cases of avian influenza A(H7N9) virus infection by date of hospitalisation, China, 1 February 2013–9 October 2014



The first wave of infections in 2013 is divided into two parts, before and after the announcement of human cases on 31 March 2013 because of the potential for under-ascertainment of less severe cases in the earlier period.

0.16% to 2.8% [3]. The objective of the present study is to estimate the HFR and symptomatic CFR in the second epidemic wave, and to determine whether the severity of human infections with influenza A(H7N9) virus has changed over time.

Methods

Sources of data

All laboratory-confirmed human cases of avian influenza A(H7N9) virus infection are reported to the Chinese Center for Disease Control and Prevention (China CDC) through a national surveillance system. Case definitions, surveillance for identification of cases, and laboratory assays have been previously described [1]. Demographic, epidemiological, and basic clinical data were obtained from each confirmed case with standardised forms. An integrated database was constructed by China CDC, with detailed epidemiological information about each confirmed case of infection with influenza A(H7N9) virus reported by 9 October 2014. We used information about age, sex, place of residence, dates of illness onset, hospital admission, intensive care unit (ICU) admission, mechanical ventilation, death, and recovery or discharge.

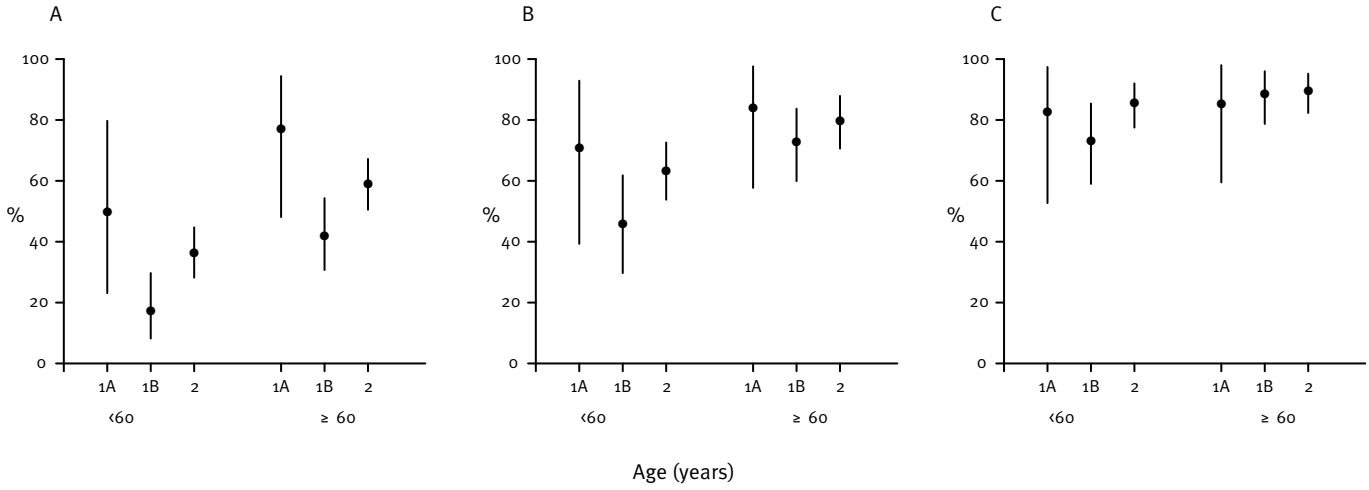
Statistical analysis

Cases were determined to be hospitalised for medical reasons (rather than solely for isolation purposes) based on routine clinical judgment, e.g. those presenting with complications such as pneumonia. A small number of cases presenting with mild respiratory symptoms did not have any complications throughout the clinical course and were hospitalised only for the purpose of isolation. Among the confirmed cases of influenza A(H7N9) virus infection that were hospitalised for medical reasons, i.e. excluding these mild cases, we estimated the risks of ICU admission, mechanical ventilation, and death. To allow for the uncertain outcomes of cases that remained in hospital on the date of analysis (9 October 2014), we used the method proposed by Garske et al., which inflates the observed fatality risk based on the time to death distribution [8]. We constructed 95% confidence intervals (CIs) using a bootstrap approach with 1,000 resamples.

To estimate the symptomatic CFR, we inferred the number of symptomatic cases based on the detection of symptomatic cases through sentinel ILI surveillance in urban areas [3]. We searched for urban areas where (i) the number of confirmed A(H7N9) virus infection cases registered by local ILI sentinels and other hospitals are both larger than one, and (ii) the number of outpatient visits at local ILI sentinels and other hospitals is available. In the spring 2013 epidemic wave, Shanghai and Nanjing (Jiangsu province) met the criteria, and in the winter 2013/14 epidemic wave the city of Shaoxing (Zhejiang province) met the criteria. In these selected urban areas, we determined the daily number of all ILI cases reported and specimens tested by ILI surveillance in each location during the relevant period to infer the number of infected individuals who would have sought medical care at ILI sentinels (N_{ILI}). We assumed that healthcare seeking behaviour of individuals with ILI associated with influenza A(H7N9) virus infection was the same as healthcare seeking behaviour of individuals with ILI associated with 2009 pandemic influenza A(H1N1) virus infection in 2009/10 in the same area of China. We used data from a nationwide serosurvey and ILI surveillance of the 2009 influenza A(H1N1) pandemic in China from June 2009 to January 2010, to estimate the proportion of individuals with symptomatic infections who sought medical care at ILI sentinels. We divided N_{ILI} by this proportion. We then estimated the symptomatic CFR in each location using the number of confirmed deaths as the numerator and the estimated

Figure 2

Estimates and 95% credibility intervals of the risk of serious outcomes among laboratory-confirmed human cases of avian influenza A(H7N9) hospitalised for medical reasons, by age and wave, China, 1 February 2013–9 October 2014



Panel A: the risk of death. Panel B: the risk of death or mechanical ventilation. Panel C: the risk of death or mechanical ventilation or intensive care unit admission.

Epidemic wave 1A: 1 February–31 March 2013

Epidemic wave 1B: 1 April–30 September 2013

Epidemic wave 2: 1 October 2013–9 October 2014

number of mild cases as the denominator. We used a Bayesian framework to estimate the symptomatic CFR, and presented the estimates with 95% credibility intervals (CrI) which have a similar interpretation to confidence intervals [9].

We examined epidemiologic time-to-event distributions using kernel density methods as previously described [2]. All statistical analyses were performed using R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) and Matlab (Mathworks Inc., Natick, Massachusetts, United States).

Results

In the first wave of influenza A(H7N9) cases in 2013, 134 confirmed cases were identified (Figure 1), of whom 124 required hospitalisation for medical reasons.

Among the hospitalised cases, the risk of serious outcomes was higher among older hospitalised cases. Furthermore, we identified higher risks of fatalities among cases hospitalised before 31 March 2013, the date when the first confirmed human cases of influenza A(H7N9) virus infection were officially announced in China (Figure 2).

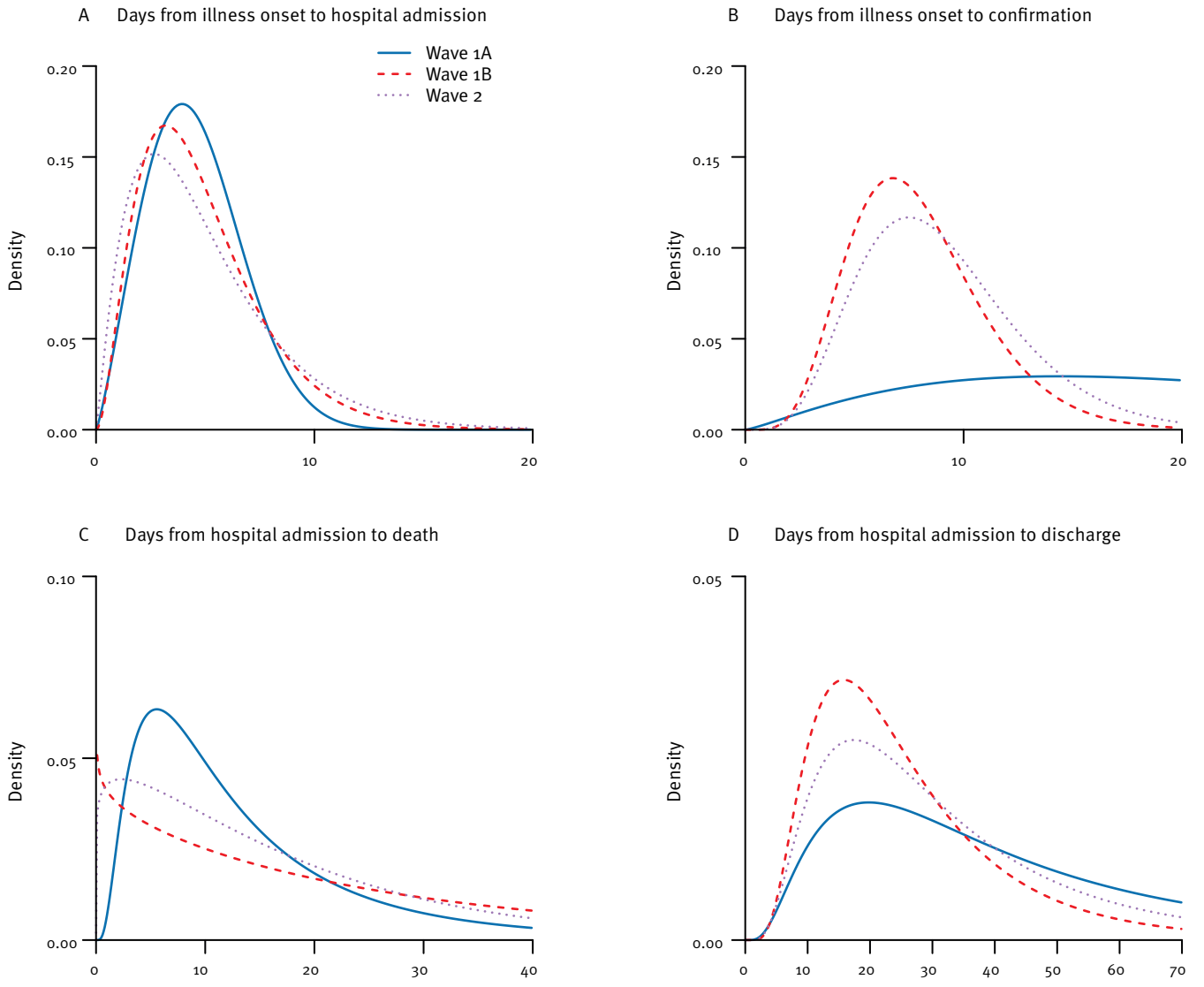
We therefore divided the first wave into two parts: wave 1A for 18 cases hospitalised before 1 April 2013, and wave 1B for 106 cases hospitalised from 1 April to 30 September 2013 (Figure 1).

In the first epidemic wave, the median age was 60 years in wave 1A and 61 years in wave 1B. Among the cases under 60 years who required hospitalisation for medical reasons, the HFR in wave 1A was 51% (95% CI: 21%–79%), significantly higher ($p = 0.039$) than the HFR of 17% (95% CI: 7.6%–30%) in wave 1B. For cases above 60 years who required hospitalisation for medical reasons, the HFR was also significantly higher ($p = 0.025$) in wave 1A (77%; 95% CI: 48%–94%) vs wave 1B (42%, 95% CI: 31%–54%). We did not identify significant differences between wave 1A and 1B in the risk of death or ventilation, or in the risk of death/ventilation/ICU admission (Figure 2).

In the second epidemic wave of influenza A(H7N9), 273 of the 306 confirmed cases required hospitalisation for medical reasons with onset dates between 1 October 2013 and 9 October 2014. The median age was 57 years (range 2–88 years). Sixty-nine percent of cases were male. Among the hospitalised cases, allowing for censoring of outcomes in five (2%) patients remain in hospital on 9 October 2014, we estimated HFRs of 36% (95% CI: 28%–45%) in cases under 60 years, and 59% (95% CI: 51%–67%) in cases aged 60 years or above. These risks were significantly higher than in wave 1B ($p = 0.019$ and $p = 0.025$ respectively). There were no statistically significant differences between the age-specific risks of death or ventilation, or death/ventilation/ICU admission in wave 2 compared to either wave 1A or wave 1B, while estimates of the risks of serious outcomes were generally lower across age groups in wave 1B compared with wave 2 (Figure 2). While the second epidemic wave occurred over a broader geographic area than the first wave, Zhejiang province was heavily affected in both epidemic waves. We

Figure 3

Comparisons of epidemiologic distributions between waves, human cases of avian influenza A(H7N9), China, 1 February 2013–9 October 2014



Wave 1A: 1 February–31 March 2013
Wave 1B: 1 April–30 September 2013
Wave 2: 1 October 2013–9 October 2014

therefore examined the risk of death among the subset of hospitalised cases in this province. Zhejiang province reported 40 cases in wave 1B and 88 cases in wave 2, and the risk of death among hospitalised cases under 60 years-old was significantly higher in wave 2 compared with wave 1B (risk ratio 7.1; 95% CI: 1.3–292; p = 0.017) and not significantly different in hospitalised cases above 60 years-old (risk ratio 1.5; 95% CI: 0.93–2.8; p = 0.099).

We examined the delays from onset to admission and identified similar patterns over calendar time, while the delay from onset to laboratory confirmation has shortened over time and in wave 2 the mean was eight days (Figure 3). Distributions of time from admission to death and from admission to discharge were similar over time (Figure 3).

We previously used information on three confirmed influenza A(H7N9) cases identified through ILI surveillance in Shanghai and Nanjing to estimate the number of symptomatic cases in the spring 2013 epidemic wave [3]. Here we also use information on four confirmed cases identified through ILI surveillance in Shaoxing in the winter 2013/14 epidemic wave, in the period from 1 January to 21 January 2014, before the closure of live poultry markets on 22 January. During the same period in Shaoxing, nine hospitalised cases had onset of illness, of whom five died. Based on these observations, we estimated that there were 3,020

Table

Estimates of the symptomatic case fatality risk, human cases of influenza A(H7N9) virus infection, China, 1 January 2013–21 January 2014

Period analysed	Geographic location	Number of confirmed deaths caused by influenza A(H7N9) virus infection	Estimated number of symptomatic A(H7N9) virus infections	Estimated risk of fatalities per 100,000 symptomatic cases
1 Jan 2013–28 May 2013	Shanghai	14	3,020 (95% CI: 900–7,800)	490 (95% CI: 170–1,800)
1 Jan 2013–28 May 2013	Nanjing (Jiangsu province)	3	5,310 (95% CI: 880–17,300)	69 (95% CI: 12–710)
1 Jan 2014–21 Jan 2014	Shaoxing (Zhejiang province)	5	5,750 (95% CI: 1,960–12,730)	100 (95% CI: 29–360)

CI: confidence interval.

(95% CI: 900–7,800) and 5,310 (95% CI: 880–17,300) cases in the first epidemic wave in 2013 in Shanghai and Nanjing, respectively, and 5,750 (95% CI: 1,960–12,730) cases in Shaoxing in the second epidemic wave in 2013/14. These estimates correspond to symptomatic CFRs of 490 and 69 in Shanghai and Nanjing respectively in the first wave, and 100 per 100,000 symptomatic cases in Shaoxing in the second wave, with wide and overlapping credibility intervals (Table).

Discussion

The resurgence of human infections with avian influenza A(H7N9) virus in a second epidemic wave in 2013/14 demonstrates the continued public health risk of this novel strain [10]. Control of the virus in animals is complicated, because the infections in poultry are asymptomatic [11]. Human-to-human transmissibility of the virus remains limited, as evidenced by the very small number of potential secondary infections identified through detailed contact tracing of confirmed cases [1,2,12-14].

We identified differences in the severity of illness of hospitalised cases in the earlier part of the first epidemic wave in 2013, with greater risk of mechanical ventilation, ICU admission and death among cases hospitalised before 31 March 2013 when the first confirmed human cases of influenza A(H7N9) were officially announced (Figure 2) [15]. One explanation for this is more timely antiviral treatment and more appropriate supportive care for cases hospitalised after 31 March 2013. Another possible explanation is detection bias in the early phase of the spring 2013 epidemic wave, where more severe cases were prioritised for repeated laboratory testing, and cases with prolonged virus shedding or higher virus shedding had a greater chance of confirmation.

In the second epidemic wave in 2013/14, we identified a significantly greater HFR compared with the latter part of the first epidemic wave in 2013 (Figure 2) and in persons under 60 years of age in Zhejiang province where cases occurred in both epidemic waves, but no difference in the symptomatic CFR (Table). It is possible that this significant difference in HFRs is due to ascertainment bias in cases in different locations at different times, even within the same province. Alternatively, the HFR could have increased, because hospitalised cases in the second epidemic wave in 2013/14 were less likely to be transferred to larger referral hospitals (Dr Enfu Chen, Chief Epidemiologist in Zhejiang Provincial CDC, personal communication, June 2014), because of changes in the virus, or because of seasonal changes in the prevalence of other pathogens that could cause secondary or co-infections and modify the severity of influenza A(H7N9) virus infections [16]. Whereas ascertainment of infections in hospitalised cases may have changed over time due to changes in awareness and testing capacity, the ascertainment of influenza A(H7N9) cases through the established sentinel ILI network should have remained more stable over time.

Large population-based serological studies in affected areas would permit assessment of severity with a denominator of infections, rather than cases of symptomatic disease or hospitalisation, and infection-based severity measures could be less susceptible to biases due to differential healthcare seeking behaviours or diagnostic capacity [3,7]. To date, few serological studies have been reported and such analyses are not yet possible [17-19].

Our estimates of the risks of serious outcomes in hospitalised cases are limited by the potential for under-ascertainment of cases, due to lack of access to laboratory testing in some areas, and the potential for imperfect sensitivity of laboratory testing for the A(H7N9) virus [20,21]. While we accounted for unknown final status of cases that remain hospitalised in our analysis, the eventual estimates may change slightly once all outcomes are known. It is challenging to estimate the symptomatic CFR based on a small number of confirmed cases with milder disease identified through sentinel ILI surveillance, and our estimates are dependent on the assumptions that coverage of the sentinel system was similar in 2013/14 compared with 2009, and that healthcare seeking behaviours for ILI were similar whether illness was caused by influenza

A(H7N9) virus or the 2009 pandemic influenza A(H1N1) virus [3]. In addition, the estimation of sCFR were based on data from geographic locations in which influenza A(H7N9) virus infections were identified through sentinel ILI surveillance, and a more comprehensive analysis could also incorporate data on ILI surveillance in other areas.

In conclusion, it remains important to assess the severity of human infections with influenza A(H7N9) virus, as part of ongoing risk assessment of this virus. While the overall picture is that the severity of human infections has not substantially changed (Table), we found some evidence that the HFR was higher in the second epidemic wave in 2013/14 (Figure 2). Our results again highlight that many influenza A(H7N9) virus infections can cause mild disease [3,5,6] and that the risk of death among laboratory-confirmed cases is a misleading measure of severity. If another epidemic of human infections with influenza A(H7N9) virus occurs in the winter of 2014/15, proactive control measures on the poultry-human interface may be preferable to reactive measures [10,22-24]. Comprehensive surveillance of avian influenza virus infections in animals and humans is essential in order to monitor risk and guide the use of control measures.

Acknowledgements

We thank staff members at county-, district-, prefecture-, and provincial- level CDCs at the provinces where human cases of influenza A(H7N9) virus infection occurred for providing assistance with field investigation, administration and data collection.
This study was funded by the United States National Institutes of Health (Comprehensive International Program for Research on AIDS grant U19 AI51915), the China-United States Collaborative Program on Emerging and Re-emerging Infectious Diseases, grants from the Ministry of Science and Technology, China (2012 ZX10004-201), the Harvard Center for Communicable Disease Dynamics from the National Institute of General Medical Sciences (grant no. U54 GM088558), the Area of Excellence Scheme of the Hong Kong University Grants Committee (grant no. AoE/M-12/06), and the Beijing Science and Technology Planning Project of Beijing Science and Technology Commission (Z131100005613048). The funding bodies had no role in study design, data collection and analysis, preparation of the manuscript, or the decision to publish.

Conflicts of interest

GML has received consulting honoraria from Janssen Pharmaceuticals. BJC reports receipt of research funding from MedImmune Inc. and Sanofi Pasteur, and consults for Crucell NV.

Authors' contributions

Hongjie Yu and Benjamin J Cowling designed the study. Luzhao Feng, Joseph T. Wu, Xiaoqing Liu, Peng Yang, Tim K. Tsang, Hui Jiang, Peng Wu, Juan Yang, Vicky J. Fang, Ying Qin, Eric H. Y. Lau, Ming Li, Jiandong Zheng, Zhibin Peng, Yun Xie, Quanyi Wang, Zhongjie Li, Gabriel M. Leung and George F. Gao collected data. Luzhao Feng, Joseph T. Wu, Tim K. Tsang, Peng Wu, Vicky J. Fang and Eric H. Y. Lau analysed data. Benjamin J Cowling wrote the first draft and all authors contributed to review and revision and have seen and approved the final version.

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The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: Results of a second structured survey

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Citation style for this article:
Grundmann H, Schouls LM, Aanensen DM, Pluister GN, Tami A, Chlebowicz M, Glasner C, Sabat AJ, Weist K, Heuer O, Friedrich AW, on behalf of the ESCMID Study Group on Molecular Epidemiological Markers and the European Staphylococcal Reference Laboratory Working Group. The dynamic changes of dominant clones of Staphylococcus aureus causing bloodstream infections in the European region: Results of a second structured survey . Euro Surveill. 2014;19(49):pii=20987. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20987

Article submitted on 19 February 2014 / published on 11 December 2014

Staphylococcus aureus **is one of the most important human pathogens and meticillin-resistant *S. aureus* (MRSA) presents a major cause of healthcare- and community-acquired infections. This study investigated the spatial and temporal changes of *S. aureus* causing bacteraemia in Europe over a five-year interval and explored the possibility of integrating pathogen-based typing data with epidemiological and clinical information at a European level. Between January 2011 and July 2011, 350 laboratories serving 453 hospitals in 25 countries collected 3,753 isolates (meticillin-sensitive *S. aureus* (MSSA) and MRSA) from patients with *S. aureus* bloodstream infections. All isolates were sent to the national staphylococcal reference laboratories and characterised by quality-controlled *spa* typing. Data were uploaded to an interactive web-based mapping tool. A wide geographical distribution of *spa* types was found, with some prevalent in all European countries. MSSA was more diverse than MRSA. MRSA differed considerably between countries with major international clones expanding or receding when compared to a 2006 survey. We provide evidence that a network approach of decentralised typing and visualisation of aggregated data using an interactive mapping tool can provide important information on the dynamics of *S. aureus* populations such as early signalling of emerging strains, cross-border spread and importation by travel.**

Introduction

Staphylococcus aureus is one of the major causes of bacterial infection in humans [1]. Infections occur in the community or in healthcare settings, predominantly following acquisition from mainly human sources. In Europe, meticillin-resistant *S. aureus* (MRSA) are predominantly acquired in healthcare settings and represent a major challenge to the control of antibiotic resistance in hospitals. MRSA has therefore become the currency with which the success of infection control initiatives is measured at health systems level [2]. *S. aureus* can also acquire particular virulence traits and has been responsible for major outbreaks of toxin-mediated disease in the community [3]. At the same time, *S. aureus* evolves gradually by successive acquisition of syntenic changes of largely unaltered core genomes. It is therefore possible to describe transmission and the consecutive spread of bacteria by genetic characterisation of highly polymorphic sites within the core genes of all isolates [4]. The importance of *S. aureus* as a human pathogen, i.e. its potential to cause large-scale outbreaks in healthcare settings and in the community, and its predominantly clonal population structure, calls for a monitoring tool that scans the distribution and spread of clones of particular public health importance over larger temporal and spatial intervals through repeated surveys. Such a tool is suited to inform public health and infection control personnel of impeding health threats.

We therefore continued with a Europe-wide initiative to explore and define any dynamic changes in the distribution and spread of clones of *S. aureus* in European hospitals five years after an initial survey was carried out in 2006 [5]. We also addressed a request from the European Centre for Disease Prevention and Control (ECDC) to explore the usefulness of integration of molecular typing data with epidemiological and clinical data at a European level.

Methods

spa typing

Molecular typing for epidemiological purposes utilises highly discriminatory genetic markers that characterise human pathogens allowing the identification of isolates that are distinct versus those that are closely related due to recent common ancestry. The *spa* locus of *S. aureus* codes for Protein A, a species-specific gene product known for its IgG binding capacity. This locus is highly polymorphic due to an internal variable region of short tandem repeats which vary not only in number but also due to nucleotide substitutions within individual repeat units [6]. DNA sequences of the *spa* gene therefore provide portable and biologically meaningful molecular typing data that have demonstrated their utility for macro- and micro-epidemiological purposes from surveillance through to outbreak investigations at various geographical levels [7,8].

Capacity building

During annually repeated workshops organised for technical personnel from European Staphylococcal Reference Laboratories (SRL), participants receive hands-on training in *spa* typing and data analysis according to a standard protocol using a purpose-designed software tool StaphType (Ridom GmbH, Würzburg, Germany) [8]. Proficiency testing was carried out by mailing each SRL five well-characterised *S. aureus* isolates and five sequence chromatograms (trace files) of known *spa* types as described previously [9,10]. All laboratories participating in the structured survey described here fulfilled quantifiable quality criteria which consisted of an unambiguous base-calling for all sequenced nucleotides for both forward and reverse sequencing runs of the test panel.

Structured survey

A protocol was agreed by all participating SRLs in June 2010. Using the same network of sentinel laboratories, this by and large followed the sampling frame deployed of the first structured survey carried out in 2006 [5]. Briefly, European SRLs were asked to approach sentinel hospital laboratories which already participated in the previous survey and which provide microbiological diagnostic services for a geo-demographically representative sample for their national patient population. Between January and July 2011, these laboratories were asked to submit the first five consecutive MSSA and MRSA isolates from individual patients with blood stream infection, from each hospital the laboratories served. If, due to low incidence, five MRSA isolates could not be obtained within these six months, laboratories were entitled to make up their quota of 10 isolates by submitting additional MSSA isolates. For small countries with only one laboratory, such as Cyprus and Malta, more than 10 samples were accepted within this sampling period. Isolates were dispatched by the participating laboratories to the SRLs and, whenever possible, accompanied by additional information, including sample number, date of isolation, demographic details (such as age and sex), epidemiological context (hospital-acquired if disease onset was more than 48 hours after admission, or community-onset for other cases), antibiotic resistance to isoxazolylpenicillin (i.e. oxacillin) or cefoxitin. SRLs confirmed MRSA by *mecA* PCR or determination of minimum inhibitory concentration for oxacillin together with PBP2a agglutination. Discrepancies between genotype or agglutination assay and susceptibility test were scored as inconclusive phenotypes. Additional information could be uploaded to the database and web application if available. This consisted of all-cause mortality 14 days after isolation of the initial bloodstream isolate. All SRLs preserved the isolates in strain collections and performed *spa* typing according to the standard protocol, uploaded the sequence information and made this available by synchronisation with the central Ridom SpaServer (www.spaserver.ridom.de) curated by SeqNet.org at the University Medical Center Groningen, the Netherlands [10,11]. Currently there are more than 13,000 *spa*-types and 630 repeat units stored on the SpaServer.

Epidemiological and typing data were communicated in parallel to a central purpose-designed structured query language (SQL) database at the Netherlands' National Institute for Public Health and the Environment (RIVM). For each local laboratory, SRLs also provided the postal address and decimal Cartesian coordinates for automatic geolocation. All data were anonymised and collected in accordance with the European Parliament and Council decision for the epidemiological surveillance and control of communicable disease in the European community [12,13]. Ethical approval and informed consent were thus not required.

Data analysis and geographical illustration

All data were inspected for inconsistencies and analysed on a country-by-country basis and returned to SRLs for feedback, clarification of inconsistencies and final approval in July 2012. After final approval, data were analysed using Stata version 11.0 (College Station, Texas, USA) using Pearson chi-squared test and Fischer's exact test for proportions and Student t-test for continuous variables. Quantitative differences with the 2006 survey were reported as results. The index of diversity (ID) is an unbiased measure of the probability of drawing two different *spa* types given the distribution of *spa* types in the sample. The 95% confidence intervals (CIs) were calculated as described previously [14]. Multilocus sequence typing (MLST) sequence types were extrapolated from the *spa* type as per the Ridom SpaServer. Cartesian coordinates were used for geolocation and plotting on Google Maps using the geocoding facility at www.spatialepidemiology.net [15]. The web application SRL-Maps (http://www.spatialepidemiology.net/SRL-Maps2) was developed to interrogate the data based on mapping of laboratory locations.

Figure 1

Geographical location of laboratories that contributed to survey of meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *S. aureus* isolates, 2011

Results

Summary statistics

Between January and July 2011, laboratories from 25 European countries participated in this survey. These included 22 European Union (EU) Member States (Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Malta, Netherlands, Poland, Portugal, Romania, Slovenia, Spain, Sweden, United Kingdom), two European Economic Area (EEA) countries (Iceland and Norway) and Switzerland. For the United Kingdom, Scotland participated on its own behalf, whereas England, Northern Ireland and Wales all referred isolates to the same reference centre in England. Two countries (Cyprus and Malta) took the deliberate decision to carry out the necessary investigations in partner (twinning) laboratories, as the sample volume would not otherwise have justified the necessary investment for sequencing equipment. Since they were also allowed to submit a larger quota, they submitted 34 and 20 isolates respectively.

Altogether, 350 laboratories (Figure 1) serving 453 hospitals submitted data for 3,753 *S. aureus* isolates from patients with bloodstream infections, isolated during the six-month investigation period. Table 1 gives a summary overview of the number of participating

Table 1

Summary overview of participating laboratories, hospitals, number of invasive isolates of meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *S. aureus* and *spa* types by country, 25 European countries, 2011

Country	Number of laboratories	Number of hospitals	Number of isolates	MSSA	MRSA[a]	Number of spa types MSSA	Number of spa types MRSA	Number not typable	Percentage non-typable
Belgium	17	17	133	76	57	53	25	3	2.2
Bulgaria	11	11	46	33	13	25	8	0	0.0
Cyprus	1	1	34	20	14	17	10	1	2.9
Czech Republic	20	20	144	95	49	63	9	0	0.0
Denmark	16	58	288	276	10	150	7	2	0.7
Finland	17	43	173	163	10	89	5	2	1.2
France	34	34	286	166	120	98	31	3	1.0
Germany	10	26	161	100	61	62	22	0	0.0
Greece	1	13	85	47	38	33	15	0	0
Hungary	13	13	125	74	51	39	13	1	0.8
Iceland	1	1	9	7	2	6	2	0	0.0
Ireland	21	27	193	117	76	68	21	13	6.7
Italy	23	23	200	103	97	61	36	0	0.0
Latvia	8	8	36	33	3	17	2	1	2.8
Malta	1	1	20	10	10	10	7	0	0
Netherlands	2	16	138	130	8	77	5	7	5.1
Norway	22	n.d.	84	80	4	61	3	0	0.0
Poland	49	52	391	312	79	136	25	0	0.0
Portugal	1	19	195	99	96	52	23	0	0.0
Romania	5	5	37	15	22	13	7	0	0.0
Slovenia	10	13	164	142	22	40	3	9	5.5
Spain	1	30	129	35	94	30	27	0	0.0
Sweden	23	n.d.	225	215	10	114	10	1	0.4
Switzerland	6	6	60	46	14	39	9	1	1.7
United Kingdom: England, Northern Ireland and Wales	16	16	164	108	56	63	25	7	4.3
United Kingdom: Scotland	21	n.d.	233	119	114	69	35	0	0.0
Total	**350**	**453**	**3,753**	**2,621**	**1,130**	**720**	**228**	**51**	**1.4**

MSSA: meticillin-sensitive *Staphylococcus aureus*; MRSA: meticillin-resistant *S. aureus*; N.d.: not determined.

[a] Note that the number of MRSA does not reflect a prevalence or occurrence in particular countries as the protocol asked for submission of the first five isolates of each phenotype.

laboratories and hospitals, isolates and *spa* types submitted by country. The combined collection consisted of 2,621 (69%) MSSA and 1,130 (31%) MRSA. Two isolates had an inconclusive resistance phenotype. One was *spa*-type t127 and the other *spa* non-typable.

A total of 861 different *spa* types were discerned, of which 720 were MSSA and 228 MRSA. Of these, 87 *spa* types were shared between MSSA and MRSA. When compared with results obtained during 2006, the *spa* type per isolate ratio remained at 0.23, indicating a similar sample diversity in both surveys. There were 51 isolates (out of a total of 3,753; 1.4%) which were *spa* non-typable. Typability of isolates ranged from 93.3% to 100% depending on the country.

Bloodstream infections with *S. aureus* occurred at an older age (median 68 years, Table 2) and predominantly in men, which is in accordance with previous findings [5,16]. The proportion of isolates from men was higher among MRSA than MSSA bloodstream infections ($p=0.03$). The median age at infection with MRSA was three years older than for infection with with MSSA. Compared with 2006 data, the age distribution for both MSSA and MRSA in 2011 had shifted slightly to older age groups ($p<0.001$).

In 2011, data on all-cause mortality 14 days after index blood culture were available for 65.5% of all cases. Overall all-cause mortality was 19.4% (477/2,458). There was a difference between MRSA and MSSA in

Table 2

Staphylococcus aureus isolated from patients with blood stream infections, 25 European countries, comparison of 2006 and 2011 data

	2011					2006					p values comparing 2011 with 2006[d]		
	n[a]	MSSA	MRSA	Total/ overall[b]	p value[c]	na	MSSA	MRSA	Total/ overall[b]	p value[c]	MSSA	MRSA	overall
Frequency (%)	3,753	2,621 (69.9)	1,130 (30.1)	3,751 (100.%)	-	2,890	1,923 (66.5)	967 (33.5)	2,890 (100%)	-	0.004	0.004	-
Median age (IQR)	3,753	67 (52–78)	70 (57–80)	68 (54–79)	<0.001	2,836	63 (46–75)	69 (55–78)	66 (49–76)	<0.001	<0.001	0.190	<0.001
Male sex (%)	3,702	1,572 (60.9)	723 (64.7)	2,295 (62.0)	0.029	2,862	1,159 (60.8)	606 (63.3)	1,765 (61.7)	0.2	0.994	0.504	0.768
All-cause mortality after 14 days (%)	2,458	289 (17.1)	188 (24.4)	477 (19.4)	<0.001	1,838	153 (13.2)	141(20.8)	294 (16.0)	<0.001	0.004	0.107	0.004
Hospital acquisition (%)	2,863	831 (44.0)	649 (66.6)	1,480 (51.7)	<0.001	2,322	777 (51.6)	585 (71.7)	1,362 (58.7)	<0.001	<0.001	0.020	<0.001
Number of *spa* types[e]	3,702	720	228	862[f]	-	2,850	565	155	660 f	-	-	-	-
Number not typable	3,753	41 (1.6)	9 (0.8)	51 (1.4)	0.060	2,890	27 (1.4)	13 (1.3)	40 (1.4)	0.9	0.660	0.220	0.930
Index of diversity (95% CI)	3,688	0.986 (0.983 – 0.987)	0.942 (0.933 – 0.947)	0.985 (0.982 – 0.984)	<0.05[g]	2,850	0.985 (0.983 – 0.987)	0.940 (0.933 – 0.947)	0.983 (0.982 – 0.984)	<0.05 g	-	-	-

MSSA: meticillin-sensitive *Staphylococcus aureus*; MRSA: meticillin-resistant *S. aureus*; IQR: interquartile range; CI: confidence intervals.

[a] Number of isolates for which information was available for each variable. In 2011, two isolates had undetermined MRSA status.
[b] Total number of isolates with an MSSA/MRSA status and data from the considered variable.
[c] p-value for the comparison of MSSA with MRSA.
[d] p-value comparing each of the three variables from 2011 with its counterpart from 2006 (e.g. MSSA 2011 with MSSA 2006).
[e] Number of typeable isolates: MSSA= 2,580 and MRSA=1,121.
[f] Total number of *spa* types includes 85 *spa* types that contain both MSSA and MRSA in 2011 and 60 *spa* types in 2006.
[g] Deduced from non-overlapping 95% confidence intervals.

Table 3

The 20 most frequent spa types and multilocus sequence typing types among meticillin-sensitive *Staphylococcus aureus* and meticillin-resistant *S. aureus* isolates collected in 25 European countries in 2011

MSSA						MRSA					
Rank	spa type	Multilocus sequence type[a]	Frequency	%	Cumulative %	Rank	spa type	Multilocus sequence type[a]	Frequency	%	Cumulative %
1	t091	ST7	138	5.3	5.3	1	t032	ST22	202	17.9	17.9
2	t084	ST15	124	4.7	10.0	2	t003	ST225	99	8.8	26.6
3	t002	ST5	121	4.6	14.6	3	t008	ST8	95	8.4	35.0
4	t015	ST45	98	3.7	18.4	4	t002	ST5	87	7.7	42.7
5	t008	ST8	97	3.7	22.1	5	t067	ST125	50	4.4	47.2
6	t012	ST30	90	3.4	25.5	6	t041	ST228	24	2.1	49.3
7	t127	ST1	83	3.2	28.7	7	t777	ST5	21	1.9	51.2
8	t021	ST30	50	1.9	30.6	8	t018	ST36	20	1.8	52.9
9	t065	ST45	38	1.4	32.1	9	t022	ST22	20	1.8	54.7
10	t026	ST45	34	1.3	33.4	10	t037	ST239	19	1.7	56.4
11	t005	ST22	33	1.3	34.6	11	t127	ST1	18	1.6	58.0
12	t230	ST45	32	1.2	35.9	12	t747	ST22	17	1.5	59.5
13	t216	ST59	28	1.1	36.9	13	t044	ST80	15	1.3	60.8
14	t056	ST101	27	1.0	38.0	14	t2357	ST22	15	1.3	62.1
15	t148	ST72	25	1.0	38.9	15	t024	ST8	14	1.2	63.4
16	t024	ST8	23	0.9	39.8	16	t740	ST45	12	1.1	64.4
17	t346	ST15	23	0.9	40.7	17	t515	ST22	12	1.1	65.5
18	t571	ST398	23	0.9	41.5	18	t6057	ST22	11	1.0	66.5
19	t701	ST8	23	0.9	42.4	19	t030	ST239	9	0.8	67.3
20	t189	ST188	21	0.8	43.2	20	t014	ST225	9	0.8	68.1
Other	-	-	1,489	56.8	100.0	other	-	-	361	31.9	100.0
Total			**2,621**	**100**		**Total**			**1,130**	**100**	

MLST: multilocus sequence typing; MSSA: meticillin-sensitive *Staphylococcus aureus*; MRSA: meticillin-resistant *S. aureus;* %: percentage.
[a] Predicted from *spa* typing data.

terms of all-cause mortality: 17.1% of patients with MSSA infections died, compared with 24.4% of patients with MRSA (p<0.001). This difference was also identified in 2006 and is explained by various confounders that put MRSA patients at a higher risk of dying than those with MSSA infections [17]. Overall, there were more patient deaths in 2011 compared with the 2006 survey (16%, p=0.004). Although observed for both MSSA and MRSA infections, this trend was only significant for MRSA infection (p=0.004). Whether this difference indicates an evolution towards more virulence or changes in host factors such as the increase in age is something that cannot be determined from this dataset.

Disease onset occurred in the community for 56% of MSSA and 33.4% of MRSA infections, indicating that MRSA remains predominantly hospital-acquired. But there was a significant increase in the proportion of cases with community onset compared with the previous survey (in 2006 48.4% of MSSA infections had community onset, p<0.001; for MRSA in the same year it was 28.3%, p=0.02). A comparison of the most prevalent *spa* types among hospital-acquired MRSA (HA-MRSA) and community-onset isolates (CO-MRSA) revealed little difference (not shown). In the 2011 sample, the five top ranking *spa* types comprised 52% and 45% of all HA-MRSA and CO-MRSA respectively.

The high overall diversity (ID=0.985) is indicative of the good discriminatory ability of *spa* typing but, as with the 2006 sample, there has been a significant difference between MSSA and MRSA as a result of the oligoclonal nature of MRSA spreading through European countries.

Overall distribution of *spa* types

For MSSA, the top 20 ranking *spa* types included 43.2% of all MSSA isolates (Table 3). Importantly, there was very little difference among the first 11 ranking *spa* types between the 2011 and 2006 datasets. Only changes in rank order were observed. Ranks 12 to 20 contained four new *spa* types in 2011 (Table 3).

For MRSA, the top 20 ranking MRSA *spa* types contained 68.1% of all MRSA isolates (73.4% in 2006). There were no differences in the top six *spa* types (albeit in relative ranking). t032/ST22 now comprises 17.9% of all MRSA sampled in 2011 (up from 14.5% in 2006, p=0.036, Figure 2, Table 3). Except for t515, all ST22 related *spa* types (t032, t022, t747, t2357, t6057) have significantly increased in frequency and this lineage made up 36% of the top 20 ranking isolates in 2011, whereas in 2006 this figure was still lower at 23%. Three *spa* types have significantly decreased compared to the 2006 collection. t008/ST8, mainly found in France, decreased from 12.4% to 8.4% (p=0.003). t041/ST228 decreased from 7.4% in 2006 to 2.1% in 2011 (p<0.001). Finally, international clone t030/ST239 decreased from 2.1% to 0.8% (p=0.013).

Discussion

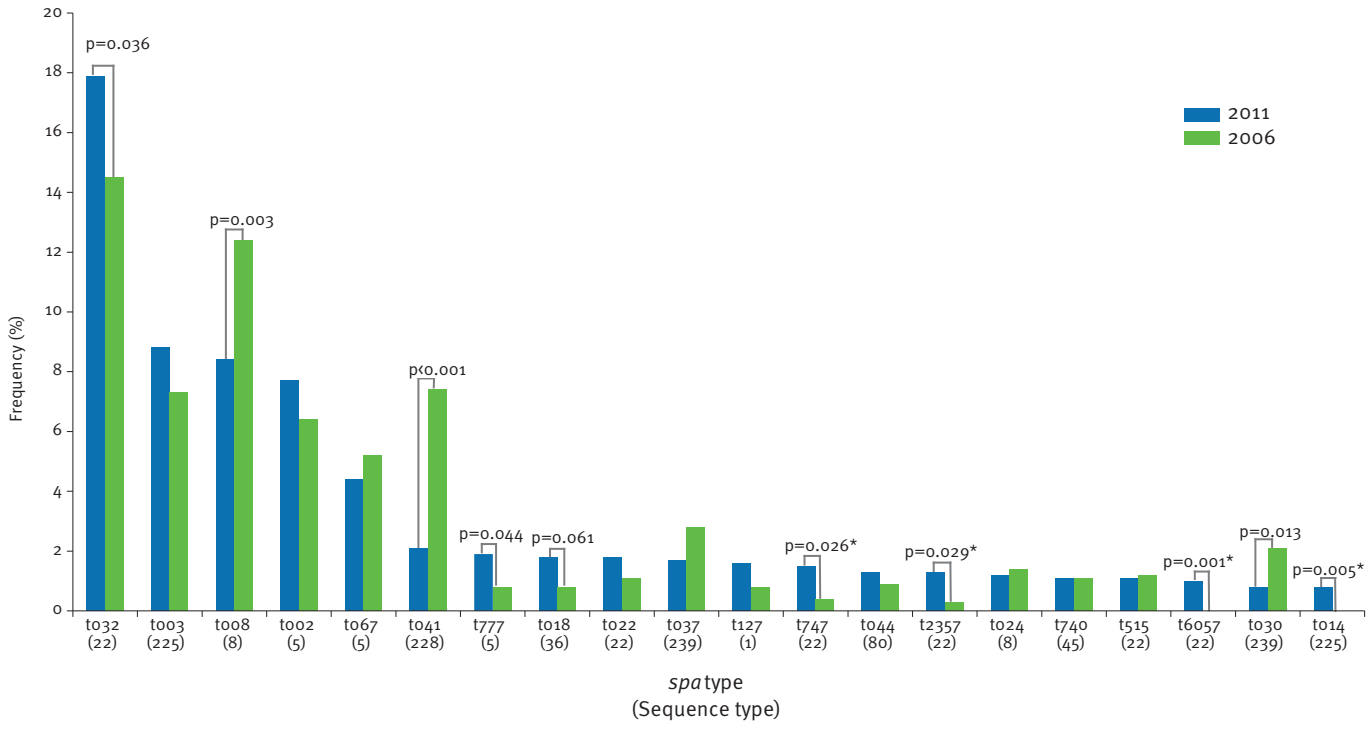
This survey represents a repetition of a previous study carried out in 2006 and was designed to investigate (i) the temporal and spatial changes of *S. aureus* clones of particular public health importance in Europe, and (ii) the feasibility and utility of integrating molecular typing data with epidemiological and clinical information at a European level.

We previously demonstrated the feasibility of creating a collaborative consortium of SRLs across Europe and alignment of *S. aureus* typing methodology in addition to harmonising processes and data format at European level [5]. The continuation of this effort has shown that collaboration between countries can be maintained over extended intervals and provide added value to the understanding of the dynamic spread of *S. aureus* while quality, consistency of molecular typing and communication improves.

The results described here are testimony to the usefulness of structured surveys to generate information for public health action in a timely and economic fashion. Repeating surveys through previously created networks of sentinel hospital laboratories allows for consistent observations about the changing epidemiology of infections caused by bacterial clones of particular public health importance. In case of *S. aureus*, these clones are responsible for community and hospital-acquired infections, and they are often resistant to a range of antibiotic compounds and circulate among patients of extended hospital referral networks in Europe. They typically have a defined geographical distribution and show a steady diffusion along hospital patient referral lines. Moreover, our results suggest that HA-MRSA is filtering into the community at an increasing rate. The proportion of community-onset infections caused by international HA-MRSA clones has increased over the last five years from 28.3% to 33.4%. This difference is significant (p<0.001) and relevant as it indicates a trend to more export of hospital-associated clones

Figure 2

Comparison of meticillin-resistant *Staphylococcus aureus spa*-type frequencies, 2011 and 2006



Brackets over the bars indicate the bars for which p values were calculated.

into the community, probably as a result of patients' shorter hospital stays.

Among MRSA isolates, a dynamic expansion was demonstrated for several *spa* types. MRSA isolates with *spa* types belonging to ST22 increased most markedly making ST22 the most critically expanding MRSA clone in Europe. This lineage (designated EMRSA-15) was first described during hospital outbreaks in England. It caused a nationwide epidemic of healthcare-associated infections in the 1990s and is still the most prevalent HA-MRSA in the UK [18]. This clone has spread from the UK and Ireland and has become abundant in Germany, Hungary, Portugal and Northern Italy. MRSA belonging to *spa* type t018/ST36 has attained a foothold in Poland and t067/ST125, abundant in Spain during the 2006 survey [19], has been causing an outbreak in hospitals in a single health district in Finland in 2011 [20]. Among MSSA, *spa* type t571/ST398 appears to be spreading in France and Belgium [21]. Our observations indicate that infections with this clone are more frequent among younger men and may be associated with higher mortality. Its MRSA counterpart has been described as an ancestral human variant [22] of the livestock-associated MRSA clone ST398, which caused outbreaks of community-acquired infections in northern Manhattan that were linked to immigrants from the Dominican Republic [23]. Conversely, a reduction of international clone ST239 consisting of the *spa* types t030 and t037 and t041/ST228 was observed. It appears that the decline of ST239 is genuine as it mainly occurred in Poland, whereas the reduction of t041/ST228 can be explained by the fact that Austria and Croatia did not participate in the 2011 survey. Both countries contributed a high proportion of this type to the 2006 dataset. This highlights the importance of consistent participation in these types of pathogen-specific surveillance initiatives and the vulnerability of networks that depend on the goodwill and enthusiasm of participants.

Limitations of this study that deserve to be addressed include a deliberate decision that was taken by the SRLs to provide only isolates from bloodstream infections. This slight deviation from the sampling frame of the previous survey may have skewed the *spa* type distribution slightly. Moreover, the fixed number of isolates that were collected from each participating centre was due to the trade-off between the desire to make the workload of SRLs predictable and manageable and the inability to precisely determine incidence and the absolute increase or decrease of *spa* types. Thus, findings generated through these types of structured surveys must be put into context of surveillance data from other European-wide initiatives such as the European Respiratory Society's (ERS-net) and/or the Healthcare-Associated Infections Surveillance Network (HAI-net). The nature of structured surveys does not allow for early warning and response as it merely provides a rather static population snapshot of the *spa* types, i.e. clones that were extant and caused bloodstream infections at the time of sampling. The value of these snapshots should not be underestimated, however, as they provide an unbiased view which can be used to identify clones of public health importance and their geographic abundance and can inform ad hoc epidemiological investigations about the dignity and geographical origin of organisms isolated during outbreaks.

The exchange of typing results using an illustrative mapping tool such as the spatialepidemiology.net website's SRL-maps provides the means to determine the reach and expansion of clones with proven success simultaneously for different countries. Initiatives such as these could lead to an improved and sustainable effort to control and eradicate emerging high-risk clones at the level of healthcare institutions once international agencies secure the sustainability for these repeated efforts.

A consistent integration of typing data with pre-existing epidemiological and or clinical data collected through other European surveillance initiatives (such as the European Antimicrobial Resistance Surveillance Network (EARS-net), European Surveillance of Antimicrobial Consumption Network (ESAC-net) or HAI-net) will, depend on the successful implementation of further alignment of sampling methodology, diagnostic procedures and of the regulatory framework across Europe. It would require a systematic and internationally accepted identification-code for hospitals and diagnostic laboratories, as well as for bacterial isolates, which are reported through different surveillance initiatives and for patients from whom these organisms were originally recovered. This would require novel regulatory approaches on the part of European national governments. Moreover, data protection and confidentiality issues would need to be resolved before such regulations could be enacted. The alternative would be a fully decentralised approach. This would require additional efforts from hospitals and laboratories to provide pertinent epidemiological and clinical information in addition to molecular typing and antibiotic susceptibility data and to report this bundled information while maintaining full confidentiality. However, as such efforts require a considerable degree of reorganisation they may seem unrealistic under the current climate of austerity. During the deliberations with the representatives of the SRLs in our study , the possibility of collecting more accurate data about source patients (epidemiology and clinical outcome) was appraised. The prevailing consensus was that this would be unrealistic given the scarcity of information provided to diagnostic laboratories by clinicians on the request forms and the inability of laboratories to fund these additional enquiries from their own budgets. It is important to note that these concerns were not raised by single members of the SRL working group, but appear to represent a common view.

In conclusion, collaborative typing initiatives are able to identify the continental spread of high-risk clones

across national boundaries and can indicate to healthcare providers the emergence of threats caused by successful and antibiotic-resistant bacteria. The geographic diffusion of antibiotic-susceptible and resistant clones of *S. aureus* can be made visible with the help of intuitive information tools such as interactive websites. This can improve the coherence of individual laboratory results and contribute to a better understanding of the population dynamic of these important pathogens. A simple integration of typing data with data from other existing surveillance efforts is, however, currently constrained by regulatory hurdles and legitimate concerns about patient data protection.

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Acknowledgements

The authors would like to like to express their gratitude to all participating laboratories and hospitals throughout the European region for sharing their isolates and demographic data for the study. We would also like to thank Roel Coutinho, Director of the Center for Infection Control at RIVM for his encouragement and supporting the organisational effort of this initiative and Carola Schinkel of RIVM and Tomorrow's Events for the organisation of the annual training and planning workshops. The support and collaboration with the ESCMID Study Group of Epidemiological Markers (ESGEM) is also gratefully acknowledged.
The study was funded by ECDC through tender and framework contract ECDC 09/033.

Conflict of interest

None declared.

Authors' contributions

ICMJE criteria for authorship read and met: HG AWF. All authors agreed with the manuscript's results and conclusions. HG, AWF and the European Staphylococcal Reference Laboratory Working Group designed the experiments and the study. HG and AT analysed the data. LS, GP, MC, CG, AS and HG collected data and/or did experiments for the study. HG wrote the first draft of the paper. KW and OH contributed to the writing of the paper. DMA developed the public domain Web-based interactive mapping tool.

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Training infection control and hospital hygiene professionals in Europe, 2010: agreed core competencies among 33 European countries

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Citation style for this article:
Brusaferro S, Cookson B, Kalenic S, Cooper T, Fabry J, Gallagher R, Hartemann P, Mannerquist K, Popp W, Privitera G, Ruef C, Viale P, Coiz F, Fabbro E, Suetens C, Varela Santos C, National representatives of the Training in Infection Control in Europe (TRICE) project. Training infection control and hospital hygiene professionals in Europe, 2010: agreed core competencies among 33 European countries. Euro Surveill. 2014;19(49):pii=20985. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20985

Article submitted on 19 November 2013 / published on 11 December 2014

The harmonisation of training programmes for infection control and hospital hygiene (IC/HH) professionals in Europe is a requirement of the Council recommendation on patient safety. The European Centre for Disease Prevention and Control commissioned the 'Training Infection Control in Europe' project to develop a consensus on core competencies for IC/HH professionals in the European Union (EU). Core competencies were drafted on the basis of the Improving Patient Safety in Europe (IPSE) project's core curriculum (CC), evaluated by questionnaire and approved by National Representatives (NRs) for IC/HH training. NRs also re-assessed the status of IC/HH training in European countries in 2010 in comparison with the situation before the IPSE CC in 2006. The IPSE CC had been used to develop or update 28 of 51 IC/HH courses. Only 10 of 33 countries offered training and qualification for IC/HH doctors and nurses. The proposed core competencies are structured in four areas and 16 professional tasks at junior and senior level. They form a reference for standardisation of IC/HH professional competencies and support recognition of training initiatives.

Introduction

There has been an increase in prevention and control activities in the field of infection control and hospital hygiene (IC/HH) in recent years owing to an increased awareness of patient safety and the considerable burden of healthcare-associated infections (HAIs) [1]. While this strengthens the role of IC/HH professionals [2], the process has not always been accompanied by a commensurate increase in the resources for IC/HH prevention and control [3]. Many reports have documented a shortage of qualified IC/HH doctors and IC/HH nurses [4-7], and there are large differences among European countries in the qualifications required to work as an IC/HH professional [8]. The need to guarantee healthcare quality standards throughout Europe, including for IC/HH, has been further stressed by the approval of the European Union (EU) directive on cross-border patient mobility [9]. In addition, topics such as patient safety, quality improvement, continuing professional development and risk management have become increasingly important and should be part of the content of contemporary IC/HH training programmes [2,10-12].

In 2006, a European survey organised by the Improving Patient Safety in Europe (IPSE) project [8] indicated a lack of national IC/HH training programmes and of professional profiles for IC/HH practitioners in many European countries. Experts from the IPSE project and representatives of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) subsequently developed a European core curriculum for training of IC/HH professionals across Europe [8].

In July 2008, at the end of the IPSE project, its activities were transferred to the European Centre for Disease Prevention and Control (ECDC) in Stockholm. In 2009, considering the importance of continued support to national IC/HH training programmes and the opportunity to build further on the outputs of IPSE, ECDC issued an open call for tender (i) to evaluate the use of the European core curriculum developed by IPSE in European countries and reassess the needs for IC/HH training in these countries, (ii) to set up a network for IC/HH training, and (iii) to propose a strategy for further support to IC/HH training in EU/EEA Member States (publication reference OJ/2009/06/16-PROC/2009/027).

The contract was awarded to the Training in Infection Control in Europe (TRICE) project under the coordination of the University of Udine, Italy. The TRICE project explored the state of the art of training IC/HH professionals in Europe, evaluated the European core curriculum together with experts from participating countries, and proposed an agreed list of core competencies to guide the standardisation of training of European IC/HH professionals [13].

The aim of this paper is to report the evolution of the status of European IC/HH activities and practitioners between 2006 and 2010 and the finalisation of the agreed European IC/HH core competencies.

Methods

The TRICE project was carried out between January and September 2010 and had two stages: a questionnaire-based survey, followed by a face-to-face consultation. Member States of the EU, the European Economic Area (EEA) and EU candidate countries were invited by ECDC to participate and were asked to designate a national representative (NR) specifically for the project.

The NR who responded to the questionnaire were nominated by the governments of each country. They were either doctors or nurses and chosen according to a profile defined by ECDC, as part of the project. In this profile it was clearly explained that the NRs had to contribute to the survey reporting the national state of the art on the topic and that they were to interact with all relevant infection control leads and other appropriate professionals in their country.

The 30 countries (33 respondents) included all EU Member States (Austria, Belgium, Bulgaria, Croatia (at the time a candidate country), Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, the United Kingdom (UK) (England, Northern Ireland, Scotland and Wales provided separate answers), one EEA country (Norway) and one EU candidate countries (Turkey). The IPSE 2006 survey lacked the participation of Cyprus, Greece, and Romania.

NRs had to be national experts on IC/HH, as they were required to document their national situations, collect data, complete questionnaires, and comment upon and approve TRICE documents. However, only nine of the 33 respondents were the same person as the one who had completed the IPSE 2006 questionnaire. For this reason, NRs were asked to validate data with other professionals in their country before returning the completed TRICE questionnaire. To facilitate this process, answers to the 2006 IPSE questionnaire were sent to the NRs together with the TRICE questionnaire. NRs were asked to contact, in case of need, IC/HH training coordinators in their country to ensure that replies were representative for the country.

Development of the core competencies for IC/HH professionals in the EU comprised the following activities:

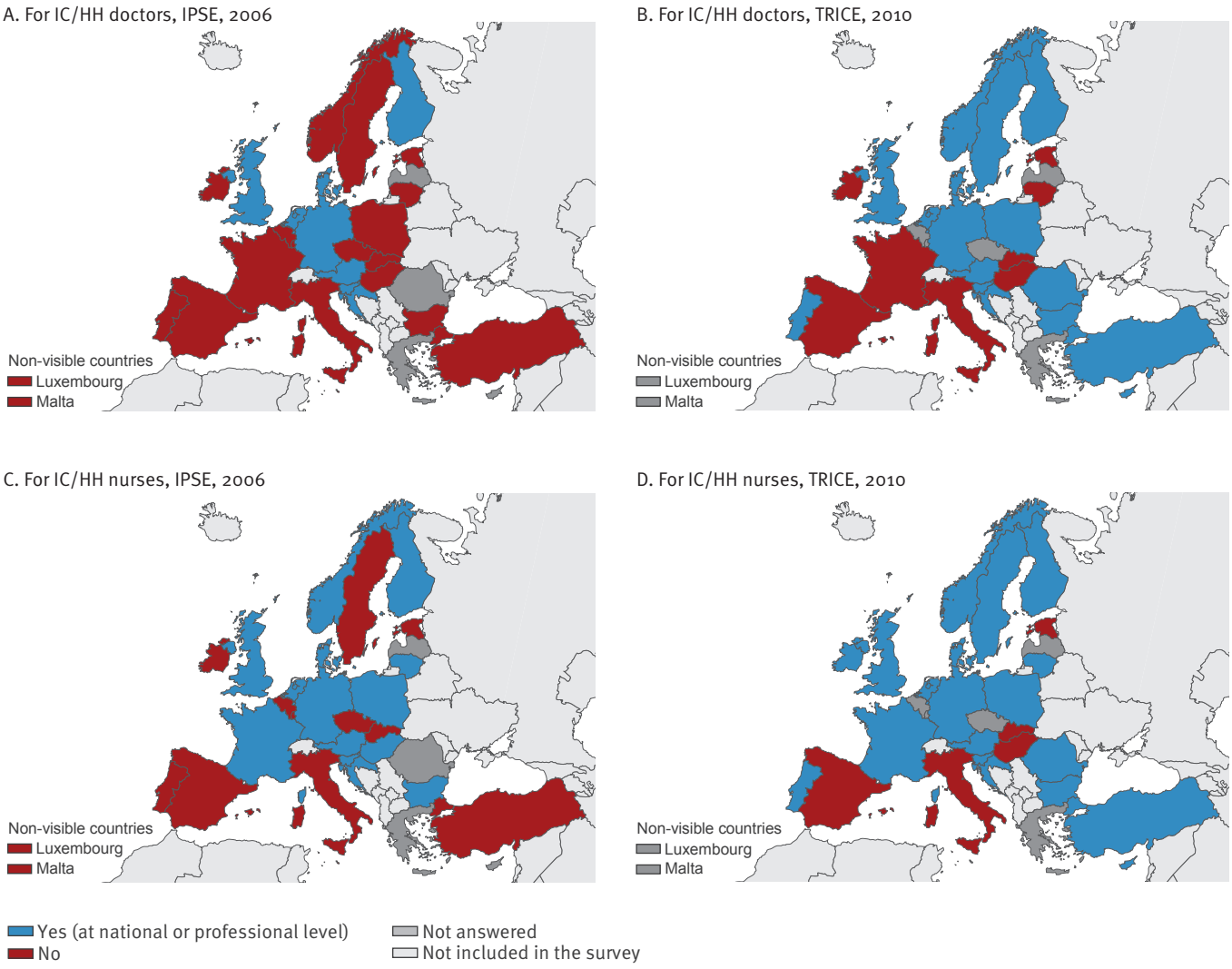
- Re-assessment by questionnaire of the existence of national programmes and courses for IC/HH training and of the current status and profile of IC/HH professionals. Countries were asked, where possible, to compare their situation in 2010 with that in 2006 (as evaluated by the IPSE project);
- Submission to the NRs of the European core curriculum for training of IC/HH practitioners developed by IPSE, together with a proposal for stratifying the core competencies in the curriculum into different levels of expertise (essential, intermediate and advanced level);
- Discussion through working groups and final general consensus of results and views at a meeting of NRs on 21 to 22 June 2010 in Udine (Italy); validation and approval by all the NRs and meeting participants of a draft of these results and views;
- Development of a final document on these core competencies, approved by the NRs.

As European countries have several different definitions, interpretations and reference frameworks relevant to competencies, the term ‘competency’, agreed by NRs, was defined as: ‘the proven ability to use knowledge, skills and personal, social and/or methodological abilities, in work or study situations and in professional and personal development’ [14].

The questionnaire was based on the previous IPSE project. It included instructions on how to fill in the responses, definitions of terms and relevant references to the IPSE project. Possible misinterpretations of the questions were addressed during data validation at a meeting of the NRs in June 2010. A pilot study was performed before launching the questionnaire. All data were individually validated by NRs twice before finalising the analysis, first to confirm that the questionnaire had been filled in and the answers had been discussed at the workshop meeting and again for the draft of the final report including tables and figures.

Figure 1

Presence of a national curriculum or programme for training of infection control and hospital hygiene doctors and nurses in European countries, 2006 (IPSE; 31 countries) and 2010 (TRICE; 33 countries)



IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; TRICE: Training needs assessment in Infection Control in Europe.

Data from the IPSE 2006 and the TRICE 2010 surveys were entered in a database and analysed with SPSS version 11 (IBM SPSS Statistics New York, United States). Data were compared with the chi-squared test accepting a value of $p \leq 0.05$ as statistical significant. To compare the ranking of the tasks performed by IC/HH professionals between the two surveys by degree of responsibility (responsible or contributor), we calculated the Spearman's rank correlation coefficient.

Results

From 2006 to 2010, the number of countries with a national curriculum or programme for IC/HH training of doctors and nurses increased from 10 of 31 responding countries to 19 of 33 responding countries for doctors (Figure 1A and B), and from 17 of 31 to 21 of 33 countries for nurses (Figure 1C and D).

For IC/HH nurses, continuous training by professional bodies was required in 11 of 33 countries, a university degree in eight countries and IC/HH specialty was required in 10 countries for nurses to play a leading role in IC/HH. From 2006 to 2010, there was a slight increase in the number of universities taking a leading role in IC/HH training both for doctors and nurses (Table 1).

More than two thirds (22/30) of European countries reported that at least one training course was available for IC/HH professionals, with 51 courses reported in total. Twenty-three of the 51 courses were run every year, six were run every second year, and for the remaining courses the frequency was not reported.

Courses provided a post-graduate diploma (where the country recognises this title for professionals) for 25 of

Table 1

Type of degree or learning leading to infection control/hospital hygiene qualification for doctors and for nurses in European countries, 2006 (IPSE; 31 countries) and 2010 (TRICE; 33 countries)

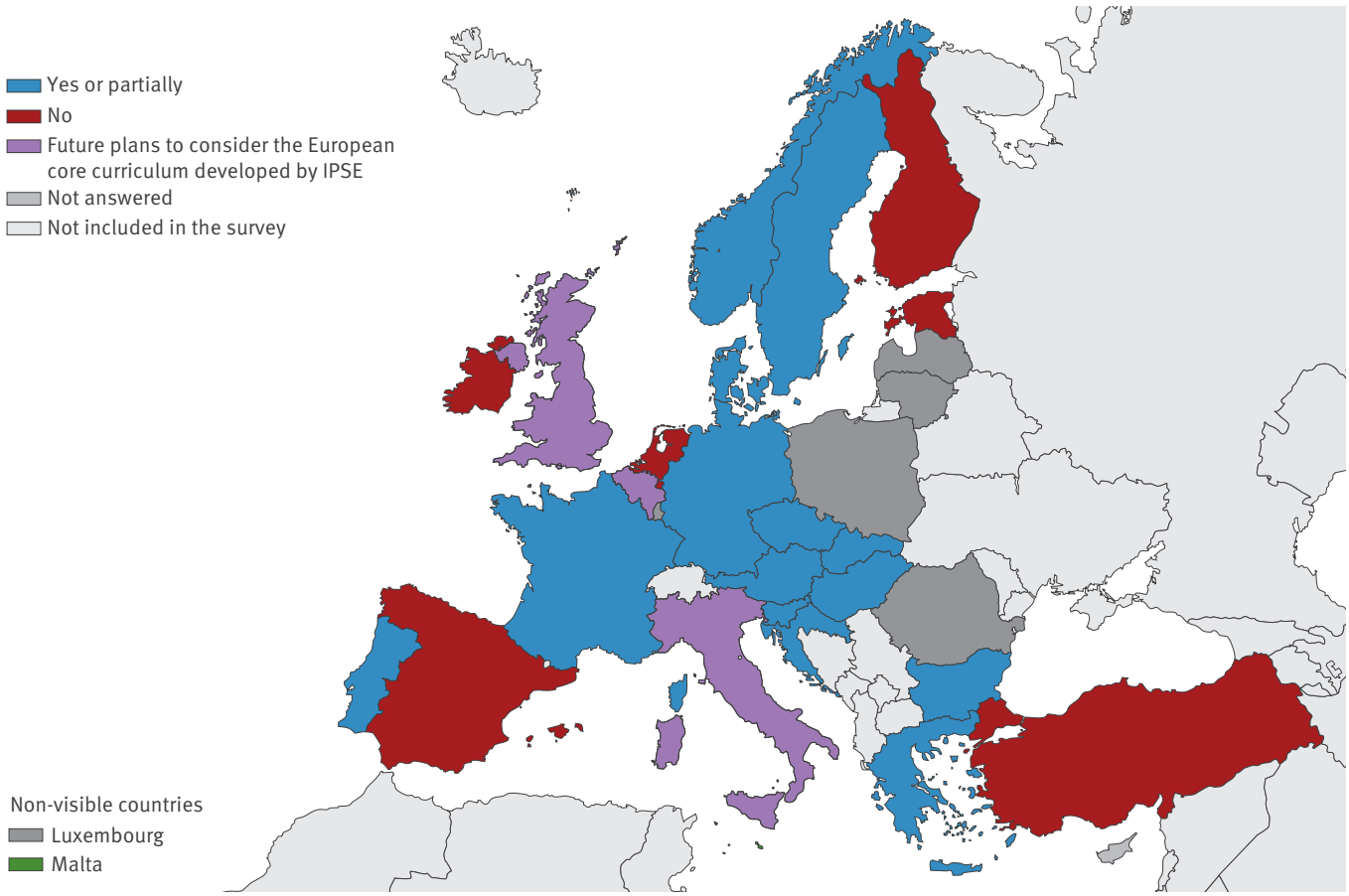
Type of degree or learning leading to IC/HH qualification[a]	Number (percentage) of countries with characteristics for			
	Doctors		Nurses	
	2006 (n=31)	2010 (n=33)	2006 (n=31)	2010 (n=33)
IC/HH specialty	4 (13%)	4 (12%)	10 (32%)	10 (30%)
IC/HH sub-specialty	2 (6%)	5 (15%)	1 (3%)	0 (0%)
Continuous training (government)	3 (10%)	5 (15%)	3 (10%)	4 (12%)
Continuous training (professional bodies)	12 (39%)	10 (30%)	11 (35%)	11 (33%)
Board certification	3 (10%)	3 (9%)	3 (10%)	3 (9%)
University degree	6 (19%)	9 (27%)	5 (16%)	8 (24%)
Other	NA	1 (3%)	NA	1 (3%)
Question not answered	12 (39%)	10 (30%)	8 (26%)	8 (24%)

IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; NA: not available; TRICE: Training needs assessment in Infection Control in Europe.

[a] More than one answer allowed.

Figure 2

Existing infection control and hospital hygiene training courses based on the European core curriculum developed by IPSE in 2006, as assessed by TRICE survey in 2010



IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; TRICE: Training needs assessment in Infection Control in Europe.

Table 2

Characteristics of the professional status (job description, professional profile and official recognition) of infection control/ hospital hygiene doctors and nurses in European countries, 2006 (IPSE; 31 countries) and 2010 (TRICE; 33 countries)

Characteristics of professional status	Number (percentage) of countries with characteristics for			
	IC/HH doctors		IC/HH nurses	
	2006 (n=31)	2010 (n=33)	2006 (n=31)	2010 (n=33)
Presence of job description	11 (36%)	17 (52%)	19 (61%)	23 (70%)
Presence of a professional profile definition	18 (58%)	28 (85%)	22 (71%)	27 (82%)
Presence of official recognition by the degree of IC/HH for doctors and nurses (any)[a]	13 (42%)	16 (49%)	20 (65%)	19 (58%)
Official recognition by health care authorities	10 (32%)	11 (33%)	18 (58%)	14 (42%)
Official recognition by universities	2 (7%)	4 (12%)	3 (10%)	5 (15%)
Official recognition by professional bodies	4 (13%)	8 (24%)	7 (23%)	8 (24%)

IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; TRICE: Training needs assessment in Infection Control in Europe.

Considered in the Table are only 30 countries that answered both surveys (2006 and 2010) in 2010.

[a] More than one answer allowed.

the 51 courses, and a post-graduation certification (in those countries where there is no formal recognition of the IC/HH profession) for 13 courses. Thirteen courses did not report a formal recognition. Nineteen of the 51 courses were recognised by the Ministry of Health and/or a State Agency, 23 by universities, and four by professional chambers (this list includes eight courses that are recognised by two institutions). The duration of the training was 100 hours or less in eight courses; 101 to 200 hours in six courses, 201 to 300 hours in 15 courses, 300 hours or more in eight courses, and one or more years in 13 courses, one course did not report the duration. Remarkably, 28 training courses reported in 2010 that they had considered the European core curriculum developed by the IPSE project and three of these courses provided distance learning programmes (Figure 2).

The questionnaire also explored the professional status of IC/HH doctors and IC/HH nurses (Table 2) with respect to the presence of a job description, professional profile definition and official recognition of an IC/HH degree. For IC/HH doctors, there was a slight increase in the percentage of countries reporting a job description from 11 of 31 in 2006 to 17 of 33 countries in 2010 ($p=0.20$) and a significant increase in the percentage of countries that provided a professional profile definition from 18 of 31 countries in 2006 to 28 of 33 countries in 2010 ($p<0.05$). However, the official recognition of the IC/HH degree did not increase between 2006 and 2010 and remained low at 16 of 33 countries for doctors and 19 of 33 for nurses (Table 2).

IC/HH teams were defined within a national programme or regulation in 27 of 33 countries in 2010, compared with 21 of 31 countries in 2006. IC/HH doctors and IC/HH nurses were confirmed as the most represented professionals in the IC/HH team (Table 3).

The initial specialisation (educational background) of IC/HH doctors was mostly microbiology, reported by 28 of 33 countries in 2010, followed by infectious diseases in 17, epidemiology in 11, public health in nine and hygiene in seven of the 33 countries.

The most frequently reported initial specialisation for registered nurses before IC/HH specialisation for nurses was graduated/certified nurse in 28 of 33 countries, followed by intensive care nurse in six, operating theatre nurse in six, anaesthesiology nurse in three and other specialised nursing background in five countries. The level of seniority required to become an IC/HH nurse was a minimum of three year of experience after qualification in 22 of the 33 countries, followed by being a senior head nurse in 10 countries.

Table 3

Healthcare professionals included in the infection control and hospital hygiene team in European countries, 2006 (IPSE, 21 countries) and 2010 (TRICE, 27 countries)

Healthcare professionals[a]	Number (percentage) of countries with characteristics for	
	2006 (n=21)	2010 (n=27)
IC/HH doctors	21 (100%)	27 (100%)
IC/HH nurses	20 (95%)	26 (96%)
Laboratory technicians	4 (19%)	6 (22%)
Environmental technicians	1 (5%)	3 (9%)
Data managers	2 (10%)	1 (3%)
Administrative support	10 (48%)	9 (33%)
Other	0 (0%)	6 (22%)

IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; TRICE: Training needs assessment in Infection Control in Europe.

[a] More than one answer allowed.

The TRICE project also reassessed the tasks for which IC/HH doctors are responsible or to which they contribute in their day-to-day practice. The most frequently reported tasks or areas of responsibility for IC/HH doctors in 2010 were: outbreak identification and investigation, analysis and feedback of IC/HH data and the development of an IC/HH programme and work plan (Table 4).

The most frequently reported tasks or areas of responsibility for IC/HH nurses in 2010 were: training of hospital employees in IC/HH and the elaboration and implementation of IC/HH procedures (Table 5). Although some significant differences in the individual tasks existed between 2006 and 2010, there was a strong correlation of the ranking of tasks by degree of responsibility (responsible or contributor) between the two surveys (Spearman's correlation coefficient 0.78, $p < 0.001$).

Besides the traditionally recognised tasks for IC/HH doctors and nurses, it appeared that the discipline of IC/HH was expanding, with the inclusion of new components such as quality and risk management.

Evaluation and finalisation of the core competencies

The results of the evaluation of the IPSE core competencies were discussed at a meeting of the NRs in June 2010. During this meeting, some changes to the IPSE core competencies were agreed and it was decided to recommend only two categories because the intermediate-level category (see Methods) was used by the NRs less frequently than the two other categories when evaluating individual competencies and was considered too complex. Hence, two main levels of practice for IC/HH training and professional development were agreed as follows:

Introductory level (junior specialist): newly appointed IC/HH staff member with little or no previous experience;
Expert level (senior specialist): IC/HH professionals who are confident and experienced, who use reasoning,

Table 4

Tasks for which infection control/hospital hygiene doctors played a role as responsible persons and or as contributors, European countries, 2006 (IPSE, 31 countries) and 2010 (TRICE, 33 countries)

Grade of responsibility	Task	Number (percentage) of countries reporting task	
		2006 (n=31)	2010 (n=33)
Responsible	Identification and investigation of outbreaks	21 (68%)	30 (91%)
	Analysis and feedback of IC/HH data	21 (68%)	25 (76%)
	Elaboration of an IC/HH programme, workplan and projects	19 (61%)	24 (73%)
	Management (implementation, follow-up, evaluation) of an IC/HH programme, workplan and projects	20 (65%)	23 (70%)
	Design of a surveillance system	15 (48%)	22 (67%)
	Elaboration of IC/HH procedures	16 (52%)	22 (67%)
	Management (implementation, follow-up, evaluation) of HAI surveillance	15 (48%)	21 (64%)
	Providing expertise in IC/HH policy	16 (52%)	21 (64%)
	Training of hospital employees in IC/HH	15 (48%)	20 (61%)
	Audits and performance evaluation of organisations and parts of them	15 (48%)	19 (58%)
	Research	13 (42%)	17 (52%)
	Implementation of IC/HH procedures	12 (39%)	17 (52%)
	Antibiotics policy	14 (45%)	17 (52%)
Contributor	Quality management	21 (68%)	25 (76%)
	Elaboration of healthcare procedures	18 (58%)	23 (70%)
	Risk management	21 (65%)	23 (70%)
	Clinical management of infected or at-risk patients	12 (39%)	22 (67%)
	Contributing to building renovation plans	NA	21 (64%)
	Contribution to construction and design of healthcare buildings	NA	20 (61%)
	Prescription of antibiotics	12 (39%)	19 (58%)
	Employee health	15(48%)	19 (58%)
	Implementation of healthcare procedures	17 (55%)	19 (58%)
	Selection of supplies or products used in the hospital	NA	19 (58%)

HAI: healthcare-associated infections; IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; NA: not available; TRICE: Training needs assessment in Infection Control in Europe.

Only the tasks reported by at least 50% of countries in 2010 are mentioned.

Table 5

Tasks for which infection control/hospital hygiene nurses played a role as responsible persons or as contributors, European countries, 2006 (IPSE, 31 countries) and 2010 (TRICE, 33 countries)

Grade of responsibility	Task	Number (percentage) of countries reporting task	
		2006 (n=31)	2010 (n=33)
Responsible	Training of hospital employees in IC/HH	18 (58%)	24 (73%)
	Elaboration of IC/HH procedures	18 (58%)	23 (70%)
	Implementation of IC/HH procedures	18 (58%)	21 (64%)
	Procedures for disinfection of medical devices	13 (42%)	21 (64%)
	Management (implementation, follow-up, evaluation) of an IC/HH programme, workplan and projects	14 (45%)	19 (58%)
	Audits and performance evaluation of organisations and parts of them	14 (45%)	18 (55%)
	Identification and investigation of outbreaks	13 (42%)	18 (52%)
Contributor	Risk management	22 (71%)	17 (52%)
	Quality management	22 (71%)	23 (70%)
	Design of a surveillance system	18 (58%)	23 (70%)
	Employee health	14 (45%)	21 (64%)
	Elaboration of healthcare procedures	20 (65%)	21 (64%)
	Research	17 (55%)	20 (61%)
	Implementation of healthcare procedures	17 (55%)	19 (58%)
	Clinical management of infected or at risk patients	14 (45%)	18 (55%)
	Providing expertise in IC/HH policy	14 (45%)	17 (52%)
	Work organisation in clinical units	10 (32%)	17 (52%)

IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; TRICE: Training needs assessment in Infection Control in Europe.

Only the tasks reported by at least 50% of countries in 2010 are mentioned.

critical thinking, reflection and analysis to inform their assessment and decision making and are able to develop and implement new solutions to problems.

The final agreed 'core competencies for infection control and hospital hygiene professionals in the European Union' are available from the ECDC website [13] and are organised in four areas (programme management, quality improvement, surveillance and investigation of HAI, and IC/HH activities) and 16 domains, each with core competencies for a junior and a senior specialist. Table 6 summarises the structure of the document.

In this context it is necessary to stress that the specification was for IC/HH specialists in acute care hospitals and that the core competencies were similar for IC/HH nurses and IC/HH doctors, including those competencies relating to antimicrobial stewardship because of the increasing numbers of prescribing nurses in European countries and their greater strategic involvement.

Discussion

The TRICE project has provided data for a comprehensive overview of the characteristics, training for professionals, tasks and related core competencies for European IC/HH professionals in 2010. It will also inform future trend analyses, as almost all countries have reported their current situation. The decision to use the questionnaire on core competencies that was used by the IPSE project in 2006, enabled us to evaluate the progress of European countries between 2006 and 2010. In particular, our results showed that IPSE initiatives and the European core curriculum developed by the project IPSE already had an impact on the creation of new national curricula for IC/HH training, on the definition of professional profiles, in particular for IC/HH doctors, and on the development or adaptation of the content of new or existing courses in European countries. Nevertheless, well-defined qualifications, standardised training pathways and agreed competencies for IC/HH professionals were still lacking in 2010. A critical point is that, in 2010, almost half of the countries still did not have an official recognition of qualifications for an IC/HH doctor or an IC/HH nurse.

The professional backgrounds of IC/HH doctors and IC/HH nurses in European countries were homogeneous, which should make the process of harmonisation of training programmes achievable. IC/HH doctors were most commonly trained in microbiology, followed by infectious diseases, hygiene and public health. In 28 of 33 countries, IC/HH nurses had a minimum of three years of experience as a graduated/certified nurse.

More than 80% of the respondents reported that a definition of the Infection Control Team existed in national programmes or regulations. The professionals most frequently included were doctors and nurses that were the focus of the survey. In future projects it would be useful to explore more in detail also the role of other professionals engaged in the infection control team.

The type of degree or learning to become an IC/HH specialist remained heterogeneous among European countries. This reinforces the need for common standards for the training of IC/HH professionals. In this context, our proposal for European core competencies for IC/HH professionals in the European Union [13] is expected to promote the standardisation of the competencies of IC/HH professionals in Europe and the design and implementation of training courses according to different national contexts and to facilitate the mutual recognition of competencies across Europe. It further serves as an opportunity for IC/HH professionals to review their own performance and plan their professional development and for healthcare institutions and organisations to evaluate their needs in terms of professional human resources and to evaluate the performance of the existing IC/HH professionals.

European core competencies are useful for countries without a national curriculum or programme to promote new training initiatives for IC/HH professionals according to European standards. For other countries, European core competencies represent an opportunity to identify gaps in their curricula and to organise IC/HH according to the different levels of expertise. These European core competencies will thus act as a point of reference for the development of IC/HH training initiatives and harmonisation of these courses and qualifications.

Professional competency grows in a continuum, where the speed and the completeness depend on several parameters. Under certain circumstances, these competencies will have to be developed very quickly. This will depend on many variables such as the expectation that IC/HH doctors or nurses will chair an IC/HH committee at an agreed time after appointment, on how long there have been IC/HH doctors or nurses in a country, on whether adequate resources are available (e.g. administrative support), on the presence and need to interact with audit/patient safety departments, and on IC/HH team members' aptitudes, preferences and previous experiences. For example, some new IC/HH professionals may have acquired considerable management experience and transferable skills from previous positions in other specialties before embarking upon their IC/HH career.

The proposed core competencies for IC/HH professionals in the European Union were developed in 2010. Since this date, the North American Certification Board

Table 6

Core competencies for infection control and hospital hygiene professionals in the European Union, by area and domain, 2010

Area	Domain	Number of competencies
Programme management	Elaborating and advocating an infection control programme	7
	Management of an infection control programme, workplan and projects	20
Quality improvement	Contributing to quality management	4
	Contributing to risk management	2
	Performing audits of professional practices and evaluating performance	9
	Infection control training of employees	5
	Contributing to research	2
Surveillance and Investigation of healthcare-associated infections	Designing a surveillance system	8
	Managing (implementation, follow-up, evaluation) a surveillance system	9
	Identifying, investigating and managing outbreaks	7
Infection control activities	Elaborating infection control interventions	14
	Implementing infection control procedures in healthcare	5
	Contributing to reducing antimicrobial resistance	8
	Advising appropriate laboratory testing and use of laboratory data	3
	Decontamination and sterilisation of medical devices	4
	Controlling environmental sources of infections	2

IC/HH: infection control and hospital hygiene; IPSE: Improving Patient Safety in Europe; TRICE: Training needs assessment in Infection Control in Europe.

Only the tasks reported by at least 50% of countries in 2010 are mentioned.

of Infection Control and Epidemiology (CBIC) has published a proposal for a conceptual model including four specific domains that represent the areas for future development of competencies: leadership, infection prevention and control, technology and performance improvement and implementation science [15]. This model does not conflict with the European approach, although it proposes an intermediate level (proficiency) between the expert and the novice. Such an intermediate level was considered by the NRs but was rejected as they expected that it may add confusion to the already heterogeneous situation in Europe. The TRICE two-level approach was considered more effective to promote standardisation of IC/HH training, and it takes into account the important diversity of duration and content of the existing IC/HH training courses in Europe. Furthermore, all participants to the TRICE agreed that a minimum of competencies for junior/introductory level was useful for both organisations and professionals and acting as a platform for personal and professional development.

The NRs also recommended that the proposed core competencies for IC/HH professionals in the European Union [13] should be seen as stages of a journey with a distinction between the two main categories (junior specialist/introductory level and senior specialist/expert level). They should be useful for the production of tools aimed at helping the development of IC/HH training courses. The introductory level should be considered appropriate for those involved in all technical/common tasks and responsibilities, whereas the expert level should apply to professionals with experience of political challenges or with new, complex, unusual or unexpected situations. The proposed core competencies should address the design of training courses developed preferably according to the Bologna process for standardisation of credits and recognition and to the standards proposed by the European Association for Quality Assurance in Higher Education [16], and they should be owned by those involved as trainers in these courses.

Conclusion

Based on the findings of the TRICE project, we consider that Europe is moving toward acknowledgement and a higher priority for HAI prevention and control. There is nevertheless room for improvement, especially in the recognition of the IC/HH degree for IC/HH professionals and in the standardisation and mutual recognition in Europe of the IC/HH training initiatives.

The future impact of the core competencies for IC/HH professionals in the European Union [13] will depend on (i) the existence of validated IC/HH training courses designed and delivered to enable the acquisition of these competencies, (ii) the widespread recognition that IC/HH doctors and IC/HH nurses need these competencies to ensure that a hospital IC/HH programme meets its goals and agreed deliverables, (iii) the existence of a sustained strategy ensuring that the core competencies are updated and reviewed at European level and owned and adopted by those running the training courses and (iv) the sustained coordination of, and support for a network for IC/HH training in Europe that will ensure the long-term commitment of institutions to the programmes.

Finally, while training of IC/HH professionals should become a priority for European countries, more emphasis should also be given to basic training in IC/HH in nursing schools and in medical universities. Ensuring that all healthcare personnel has received such basic training before starting their professional activity would certainly contribute to further improvement in the quality of healthcare throughout Europe.

Acknowledgements

The authors wish to thank the members of the pedagogic committee of Work Package 1 of the IPSE project (European Commission, DG SANCO, Project no. 790903, Grant Agreement no. 20044216) who developed the first version of the "Core competencies for infection control and hospital hygiene professionals in the European Union". Project coordination: Jacques Fabry, Josette Najjar-Pellet (Claude Bernard University Lyon 1, France); ESCMID representatives: Benedetta Allegranzi (WHO, Geneva), Jette Holt (Statens Serum Institute, Copenhagen, Denmark), Christian Ruef (Division of Infectious Diseases and Hospital Epidemiology, Hirslanden Hospital and Medical Center, Zurich, Switzerland), Nina Kristine Sorknes (Norwegian Institute of Public Health, Oslo, Norway) and Andreas Voss (Radboud University, Nijmegen, The Netherlands); Barry Cookson (Division of Infection and Immunity, University College London, United Kingdom).

Funding: The 'Training in Infection Control in Europe' (TRICE) project was funded by ECDC through a specific service contract (ECD.1840) with the University of Udine, Italy.

Conflict of interest

None declared.

Authors' contributions

Silvio Brusaferro coordinated the project. Silvio Brusaferro, Barry David Cookson, Francesco Coiz, Elisa Fabbro, Carl Suetens, Carmen Varela Santos, Smilja Kalenic, Rose Gallagher, Tracey Cooper, Jacques Fabry, Philippe Hartemann, Kerstin Mannerquist, Walter Popp, Gaetano Privitera, Christian Ruef, Pierluigi Viale designed the study, designed the questionnaire, contributed to the analyses of the results, drafted and commented on revisions to the manuscript and gave the final approval of this version. Silvio Brusaferro and Francesco Coiz conducted the statistical analysis. The National representatives of the project completed the national questionnaire, revised the manuscript and gave the final approval of this version. National representatives of the Training in Infection Control in Europe (TRICE) project

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Enhancing infectious disease mapping with open access resources

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Citation style for this article:
Pigott DM, Kraemer MU. Enhancing infectious disease mapping with open access resources . Euro Surveill. 2014;19(49):pii=20989. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20989

Article published on 11 December 2014

The Spatial Ecology and Epidemiology Group (SEEG) at the University of Oxford is currently carrying out several disease and vector mapping projects. The source code for many of these projects have been and will continue to be made openly available through our GitHub account. This archive includes a description of the R software package 'seegSDM', that allows for species distribution modelling using a boosted regression tree approach and a walkthrough tutorial. This method has been successfully applied to diseases such as dengue, ebola virus, leishmaniasis, and anopheline vectors.

The group has collated a number of globally comprehensive and up-to-date databases from published and unpublished sources. The databases compile geographically located records of occurrence, from three sources: (i) comprehensive PubMed searches, (ii) information from unpublished health surveys and entomological field studies made available by collaborators, and (iii) internet disease surveillance systems such as HealthMap. These occurrences, defined as unique spatio-temporal records of pathogen transmission or vector presence, serve as standardised input data used to generate global risk maps. Prior to modelling these databases all go through a number of technical validation steps to ensure their spatio-temporal accuracy and overall validity. This is facilitated by overlaying these occurrence records with independently derived evidence consensus maps that summarise the quality and diversity of disease information.

Disease and vector specific databases are then made openly available through online depositories which cover the diseases mentioned above. Similarly, occurrence records of the dominant vector species of malaria and more comprehensive prevalence datasets of the global distribution of *Plasmodium falciparum, Plasmodium vivax* [1] and related inherited blood disorders are downloadable through the Malaria Atlas Project. Details about collection methods, design and validation of the respective datasets can be found either in published research articles or within data descriptor publications [2–4].

Future efforts include developing the Atlas of Baseline Risk Assessment for Infectious Diseases (ABRAID), an automated mapping platform which integrates the framework described above to generate spatially comprehensive, iteratively improving, evidence based maps of disease risk at the global level for a prioritised number of infectious diseases [5].

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