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Know, treat, prevent – HIV testing, a key strategy in the prevention and control of the HIV/AIDS epidemic

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Nearly 30 years ago, human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) emerged as a new infection/disease and have since been a major concern for public health worldwide due to its associated morbidity and mortality. Considerable research and efforts have been undertaken to find and implement the best ways to prevent the spread of HIV and cure the disease AIDS. While there is still no effective vaccine, prevention programmes targeted at reducing risky behaviours have helped prevent the feared explosion of the epidemic in European Union (EU) and European Economic Area (EEA) countries. In addition, development of new antiretroviral drugs and early treatment of newly detected cases have contributed considerably to the reduction in associated morbidity and mortality since the mid-1990s. Still, there is no effective cure yet and while antiretroviral treatment is improving quality of life, there are concerns about the increasing number of HIV infections in certain groups, for example, among men who have sex with men (MSM) in several EU countries [1] and the fact that the number of other sexually transmitted infections (STIs) is also increasing in this group [1,2].

A year ago, a special theme issue of *Eurosurveillance* highlighted specific aspects of HIV and other STI in MSM. Today's issue of the journal draws attention to the latest figures on HIV/AIDS in EU/EEA countries that were released on World AIDS Day 2010 (1 December) in a report on HIV/AIDS surveillance in Europe in 2009, published by the European Centre for Disease Prevention and Control (ECDC) [3,4]. They demonstrate that in 2009, more than 4,500 newly diagnosed AIDS cases were reported by 27 EU/EEA countries; in addition, nearly 26,000 diagnosed cases of HIV infection were reported by 28 such countries. Overall, AIDS is decreasing in almost all EU/EEA countries, except in some eastern European countries, and this trend reflects the wide availability of antiretroviral treatment and care.

In the general population the rate of HIV infection remains relatively low, but infection rates are high in certain population groups, e.g. MSM, individuals from countries with generalised HIV epidemics, and

injecting drug users, with the largest increase in HIV infections among MSM.

The drivers of the HIV/AIDS epidemics are remarkably distinct between EU/EEA countries: while in many countries MSM are the most important risk group, in Bulgaria, Estonia, Latvia and Lithuania, injecting drug users are the main risk group. Knowledge about these drivers is of utmost importance in designing effective preventive strategies.

In 2009, the number of cases of HIV infection increased in 16 countries, while it decreased in only 12, when compared with 2004 data. These figures – together with the fact that an estimated 30% of infected persons are unaware of their HIV infection [5] and may not take the necessary precautions (such as practicing safer sex) to prevent transmission to others – show why HIV/AIDS remains an important public health issue and will stay in the focus of ECDC's attention.

HIV testing, early diagnosis and access to treatment are key strategies for HIV/AIDS prevention. Individuals who are unaware of their infection are at risk of progression of the disease, severe complications and possibly death from AIDS, as they cannot benefit from treatment. There is growing evidence that widespread access to treatment may reduce HIV incidence by reducing viral load at population level [6,7]. In addition, a meta-analysis showed that people diagnosed early may be less likely to transmit the virus [8].

The paper by Likitavicius and Van de Laar in this issue [3] also highlights the considerably high proportion of so-called 'late presenters' – people who present with an HIV infection that is already advanced so that the opportunity for timely access to treatment and care is missed. It reveals that HIV testing should be offered to those at risk to ensure early diagnosis and thus better prospects for reduced illness and prolonged life.

On the occasion of World AIDS Day 2010, ECDC also presented a synthesis of evidence on HIV testing, based on a literature review, and a report pointing out the need to scale up HIV testing in the EU, including

the screening of pregnant women to reduce mother-to-child transmission [9,10]. These reports show that HIV testing is an important tool to prevent further transmission and to enable early diagnosis for timely referral to treatment and care. Furthermore, the guidance describes core principles that should be considered in national testing strategies and steps to be taken when setting them up. The reports were launched during a scientific seminar at the European Parliament that was attended by many stakeholders who engaged in a lively debate demonstrating interest and support for our work. 'Know, treat, prevent' has been ECDC's motto for this year's World AIDS Day. To continue the efforts to reverse the trend of increasing numbers of HIV infections in Europe and elsewhere, concerted action is needed. In this respect it is vitally important to bring HIV testing closer to those at high risk, while simultaneously avoiding stigmatisation of and discrimination against people living with HIV/AIDS.

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HIV and AIDS in the European Union, 2009

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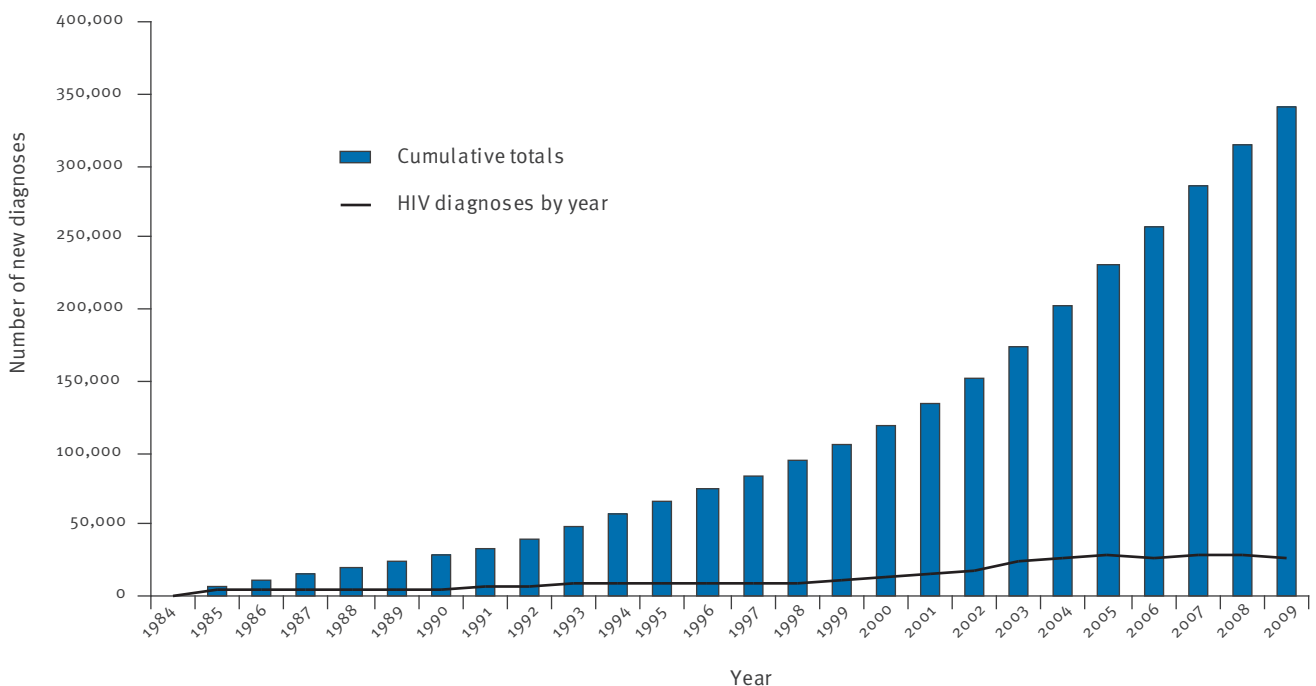
In 2009, 28 European Union and European Economic Area (EU/EEA) countries reported 25,917 newly diagnosed cases of human immunodeficiency virus (HIV). Sex among men who have sex with men was the most common transmission mode (35%) followed by heterosexual contact (24%). Overall, the number of HIV cases in 2009 increased while the number of reported acquired immunodeficiency syndrome (AIDS) diagnoses continued to decline. It is of concern that a high proportion of the patients with known CD4 cells count at the time of HIV diagnosis had a CD4 cell count below 350cells/ μ l suggesting no timely access to treatment and care.

Human immunodeficiency virus (HIV) cases in the European Union and European Economic Area (EU/EEA), 2009

In the EU/EEA, 25,917 people were newly diagnosed with HIV in 2009, reported by 28 countries, a rate of 5.7

per 100,000 population. Data was not reported from Austria or Liechtenstein. The overall rate for men was 8.3 per 100,000 male population and 3.2 for women. The highest rates of new HIV diagnoses were reported by Estonia (30.7), Latvia (12.2), the United Kingdom (UK) (10.7) and Belgium (10.3). The lowest rates (<1.0) were reported by Romania and Slovakia. Twelve per cent of new HIV diagnoses were reported in 15–24 year-old individuals and 28% were female. Sex among men who have sex with men (MSM) is the predominant reported mode of transmission in EU/EEA, accounting for 35% of the HIV diagnoses, followed by heterosexual contact (24%) when cases from countries with generalised HIV epidemics are excluded. Five per cent of HIV cases were reported among intravenous drug users (IDU). Transmission mode was unknown for 20.3% of the cases. The highest proportion of cases classified as heterosexually transmitted and originating from countries with generalised epidemics, was observed

FIGURE 1
Number of diagnosed and reported HIV infections, EU/EEA, 1984-2009



EEA: European Economic Area; EU: European Union.

in Norway (70%), Sweden (69%), Ireland (62%) and Belgium (60%).

Trends in HIV cases in the EU/EEA

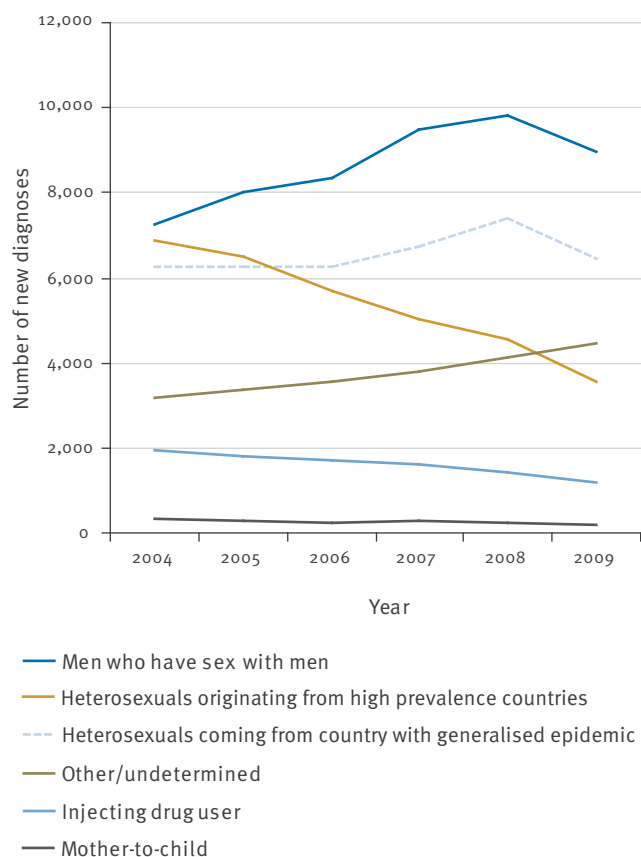
Among the 28 EU/EEA countries that have consistently reported HIV data since 2004, the rate of HIV diagnoses per 100,000 population has been relatively stable, ranging from 6.5 in 2004 to 5.7 in 2009. In recent years, more than 25,000 HIV diagnoses were reported each year, resulting in a cumulative total number of nearly 350,000 diagnoses reported since the beginning of the epidemic (Figure 1). Since 2004, the proportion of newly HIV diagnosed women decreased from 36% to 28% in 2009. HIV diagnoses have tripled in Bulgaria, Iceland and Slovakia and doubled in Hungary and Slovenia. HIV diagnoses have decreased by more than 20% in Denmark, Estonia, Italy, Luxembourg and Romania.

Since 2004, 26 EU/EEA countries have consistently reported data on transmission mode (Estonia and Poland were excluded due to inconsistent reporting). The number of new HIV diagnoses reported as heterosexually acquired decreased by 24% from 13,148

cases to 9,975 cases. The proportion of heterosexually acquired HIV diagnoses from countries with a generalised epidemic, varied from 52% in 2004 to 38% in 2009. In the same period, the number of cases among MSM increased by 24% in, from 7,263 cases to 8,974 cases and declined among IDU by 40% from 1,952 cases to 1,171 cases. The number of cases for which the transmission category was unknown increased by 40% (Figure 2). There are reporting delays for a number of countries which overall limits the ability to interpret the trends in recent years.

In 2009, a total of 4,650 cases of AIDS diagnoses were reported in 27 EU/EEA countries (no data from Austria, Liechtenstein, Sweden), representing a rate of 1.0 cases per 100,000 population. The highest rates were reported by Latvia (4.3), Estonia (2.8), Portugal (2.8), and Spain (2.3). In the EU/EEA, a decline was observed from 9,012 in 2004 to 4,650 in 2009 in all but four countries of the 27 countries reporting AIDS diagnoses consistently. An increase in AIDS diagnoses was reported only in Bulgaria from 22 cases to 30 cases (36%), Estonia 29 cases to 38 cases (31%), Latvia 89 cases to 96 cases (8%) and Lithuania 21 cases to 37 cases (76%).

FIGURE 2
HIV infection by transmission group and origin in EU/EEA countries^a 2004-2009



EEA: European Economic Area; EU: European Union.
^a Data from Austria, Estonia and Poland not included

Proportion of late presenters

Late presenters are defined as patients with a CD4 cell count below 350 cells/ μ l at time of HIV diagnosis [1]. Data on CD4 cell counts at the time of diagnosis were available for cases in 18 countries, ranging from 1.2% in Bulgaria to 87% in Spain. For 11 countries, CD4 count information was available for more than 50% of the cases (Table). Half of these were reported as late presenters, also taking into account possible reporting bias for more advanced HIV diagnoses. A slightly higher proportion of female cases (54.4%) were reported as late presenters compared with male cases (49.6%), with high heterogeneity among females across countries ranging from 33% in Cyprus and Luxembourg to 68% in Denmark. Among males, the proportion of late presenters ranged from 20% in Luxembourg to 60% in Denmark.

Among new diagnoses acquired heterosexually the proportion of late presenters ranged from 20% in Luxembourg to 67% in Slovakia, while seven countries reported more than 50% of the newly diagnosed cases as late presenters (Table). For MSM, the proportion of late presenters varied from 25% in Luxembourg to 50% in Slovenia (Table). Among IDU, the proportion of late presenters ranged from 49% in the UK to 60% in Denmark. All countries but Spain and the UK reported more than half of their cases with unknown risk factor as late presenters.

Figure 3 shows that among 10 222 cases with CD4 cell count reported, 51% were "late presenters". The largest proportion of late presenters is among individuals originating from sub-Saharan Africa and southeast/

Proportion of CD4 cell counts reported for all HIV diagnosed cases in 2009, CD4 cell counts <200 among all reported, CD4 cell counts <350 among all reported and CD4 cell counts <350 by transmission mode by reporting country, 11 EU Member States^a, 2009

Country	2009				CD4 <350 mm ³ blood in 2009																
	Number of cases with CD4 cell count	Completeness (%) CD4	Number of cases CD4 <350	% CD4 <350	Cases classified as						Unknown										
					Hetero		IDU		MSM		MTCT		Hetero		IDU		MSM		MTCT		
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Cyprus	29	76	12	41	21	42.9				8	37.5										
Czech Republic	117	75	48	41	34	64.7	4	75.0	78	28.2										1	100.0
Denmark	181	77	91	50	105	57.6	14	60.0	108	39.0										8	100.0
France	2624	54	1350	51	1367	56.8	71	53.5	916	40.9	22	40.9								245	60.8
Luxembourg	33	70	8	24	15	20.0			16	25.0										2	50.0
Netherlands	653	80	306	47	179	61.5	1	0.0	435	39.3										37	64.9
Romania	114	80	45	40	72	30.6	1	0.0												41	56.1
Slovakia	41	77	17	42	6	66.7	1	100.0	32	34.4										2	50.0
Slovenia	42	88	22	52	6	33.3			28	50.0										8	75.0
Spain	1968	87	987	50	709	58.8	152	59.2	899	42.3	7	42.9								197	47.7
United Kingdom	4365	66	2268	52	2205	62.5	89	49.4	1771	39.5	12	75.0								264	46.6

EU: European Union; IDU: injecting drug user; MSM: men having sex with men; MTCT: mother-to-child transmission.

^a Member states not included in table: Belgium, Bulgaria, Finland, Germany, Latvia, Lithuania, Portugal.

eastern Asia, followed by cases from the Caribbean and Latin America. The proportion of late presenters is slightly higher in eastern Europe compared with central and western Europe. The areas are defined in the most recent ECDC/WHO HIV/AIDS surveillance report [4].

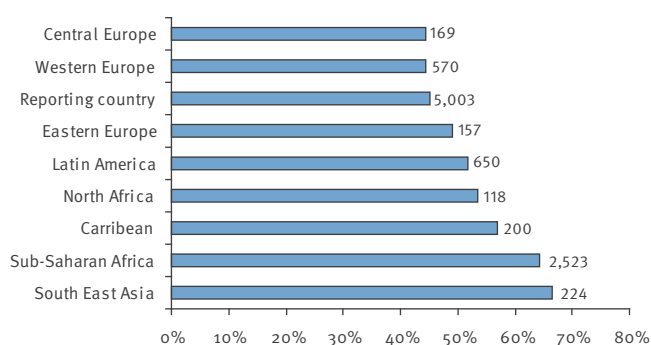
Conclusions

As in previous years, the highest proportion of the total number of new HIV diagnoses in 2009 in EU/EEA countries was reported among MSM, followed by heterosexuals. For the known transmission modes, a substantial increase was reported only in MSM. Heterosexual HIV transmission continues to be frequently reported; the proportion of cases classified as heterosexually acquired in persons originating from countries with generalised epidemics is decreasing, although it is still high in several countries. Although there is an apparent decline in the number of HIV diagnoses among IDU, injecting drug use is still the predominant transmission mode in several Eastern European countries. The transmission mode for a significant proportion of cases was reported as unknown, highlighting the importance of the improvement of surveillance data to better target public health interventions. The number of AIDS cases is decreasing in most of the countries except Bulgaria and Baltic States.

It is important to obtain more rigorous HIV surveillance data to better reflect changing epidemiological conditions. Inclusion of CD4 cell count at diagnosis provides an opportunity to interpret the data in greater depth. However, the reporting of CD4 cell counts needs to be improved as it was reported only for 40% of all new HIV diagnoses in 2009. Half of those were diagnosed as late presenters, and it appears that late presenters are more often from outside of Europe. Timely HIV diagnosis is beneficial for the patient as it decreases morbidity and mortality and reduce HIV transmission [2-3]. Many factors influence the interpretation of the data such as the stage of the epidemic in the region, migration patterns and reporting bias. However the data suggest that access to testing and treatment needs to be improved among those at risk. Recently, ECDC has launched guidance in HIV testing to support

FIGURE 3

All cases with CD4 cell count reported, proportion of late presenters by region of origin (n=10,222)



Member States in the increasing the uptake of HIV testing in Europe [4-7]

Surveillance of HIV and AIDS in Europe provides the large scale picture of the HIV epidemics within its regions and of its main characteristics and risk groups affected, which is necessary to monitor the epidemic and guide the public health response to control HIV transmission of infections. Ensuring high quality of the data is of utmost importance to follow up and achieve the objectives set up in the EU Commission communication and action plan 'Combating HIV/AIDS in the European Union and Neighbouring countries, 2009-2013'.

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A foodborne outbreak of *Salmonella* Bareilly in the United Kingdom, 2010

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We report the preliminary findings of the investigation of an outbreak of foodborne *Salmonella* Bareilly. Between August and November 2010, there were 231 laboratory-confirmed reports of *S. Bareilly* in the United Kingdom. A case-control study showed that consumption of bean sprouts was significantly associated with illness. The investigation concluded that raising public awareness to ensure the correct preparation of raw bean sprouts during cooking was the principal means of preventing further cases.

Background

Consumption of bean sprouts has previously been associated with outbreaks of *Salmonella* infection [1,2]. There was previously an outbreak of *S. Saint-Paul* infection associated with bean sprouts in the United Kingdom (UK) in 1988 [3].

Salmonella enterica subsp. *enterica* serovar Bareilly, or *S. Bareilly*, is a group C1 serovar first identified in India in 1928 [4]. In years when no outbreaks occur, on average 30 to 50 cases are reported in England and two to five cases in Scotland.

The current investigation began on 27 August 2010, when an outbreak of *S. Bareilly* was reported following a wedding reception in Greater Manchester in north-west England. An increase in the number of detected cases of *S. Bareilly* was also reported in Scotland on 2 September 2010 and more widely in England on 10 September 2010. The outbreak was first reported to the European Centre for Disease Prevention and Control (ECDC) Epidemic Intelligence Information System (EPIS) site on 14 September, providing an image of the outbreak pulsed-field gel electrophoresis (PFGE) profile. Between 14 September and 1 October, 10 EPIS members responded with no indication of co-temporal increases in findings of this serovar. One country (Ireland) reported the same profile in a case who had

visited London in September, but no further exposure data was available.

Preliminary information from cases in Scotland, from the wedding outbreak investigation, and from trawling questionnaires including questions on a comprehensive range of possible risk factors including foodstuffs, raised the hypothesis of salad leaves and/or bean sprouts as possible vehicles of infection. A matched case-control study including 34 cases meeting the case definition was conducted across the UK beginning on 21 September 2010. At the time the case-control study started there were some 40 cases who had not done the trawling questionnaire and who were not linked to the wedding outbreak.

Between the beginning of August and the 19 November 2010, there were 231 laboratory-confirmed reports of *S. Bareilly* across the UK. 10 of these were linked to the wedding in Greater Manchester.

Case-control study Definition of cases and controls

The case-control study was conducted using telephone interviews with cases of *S. Bareilly* fitting the confirmed case definition and with controls selected by sequential digit dialling based on the telephone number of the case, to match on broad geographical location of residence.

Confirmed cases were defined as persons aged 18 and over with microbiologically confirmed infection with *S. Bareilly* with a sample received at the Health Protection Agency (HPA) *Salmonella* Reference Unit or the Scottish *Salmonella*, *Shigella* and *Clostridium difficile* Reference Laboratory on or after 1 August 2010 with (i) no history of foreign travel or close contact with a case of diarrhoea in the seven days prior to the onset of illness, (ii) who did not attend the wedding in the

northwest of England associated with the point source outbreak and (iii) who had not been previously interviewed as part of the preliminary investigation.

Controls were defined as residents of England, Wales, Northern Ireland or Scotland aged 18 and over residing in the area covered by the same telephone exchange as their matched cases who had not had gastrointestinal symptoms (vomiting and/or diarrhoea) in the seven days prior to interview, and who had no history of foreign travel or of close contact with a case of diarrhoea in the seven days prior to interview. The person answering the telephone was recruited unless they were a child, in which case interviewers asked to speak to an adult. The case–control ratio was 1:2.

Interviews

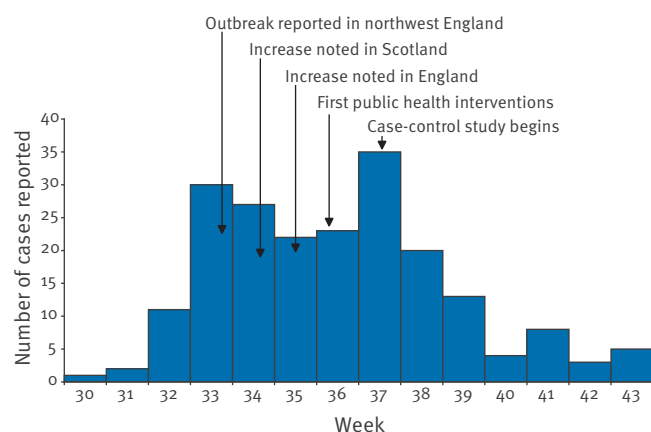
Data were collected on demographics, clinical features and outcomes, travel history, infectious contacts, and consumption of salad leaves, bean sprouts or alfalfa sprouts in the three days prior to illness (or three days prior to interview for controls). Information on travel history and infectious contacts was collected to check eligibility. After descriptive and univariate analysis, forward stepwise conditional logistic regression was conducted using the statistical software R [5]. Possible risk factors with a *p* value of <0.2 in univariate analysis were added sequentially after assessment for multicollinearity.

Laboratory investigation of food items

Samples of bean sprouts (100 g) were collected from suppliers B and C. They were examined using HPA standard methods, based on BS EN ISO 6579:2002, for the detection of *Salmonella* spp, modified to include extended incubation for 48 hours for both enrichment media (Muller-Kauffmann tetrathionate novobiocin broth and Rappaport-Vassiliadis Soya Peptone broth) and selective solid media Xylose Lysine Desoxycholate and Brilliant Green agars [6].

FIGURE

Epidemic curve of *S. Bareilly* cases in England, Wales and Northern Ireland^a, 2010 (n=213)



^aBy specimen date.

Results of case–control study

Data on 34 cases (response rate of 77%) meeting the case definition, with onset dates ranging from 13 September 2010 to 14 October 2010, and 64 eligible controls were collected. Cases had a mean age of 49 years (standard deviation 15.1 years) and 21 of 33 cases were female. A total of 32 of 34 reported diarrhoea and seven of 34 reported vomiting. Five cases had been hospitalised as a result of their infection. Cases reported a median duration of illness of 7.5 days (range 2 to 30 days).

In univariate analysis, consumption of bean sprouts, consumption of any salad leaves and age younger than 65 years were all significantly associated with illness. In the final conditional logistic regression model, only consumption of bean sprouts was significantly associated with illness (crude matched odds ratio (OR) 8.3, 95% confidence interval (CI) 1.8 to 38.7; adjusted matched OR 6.8, 95% CI 1.4 to 33.0). Of the 15 cases who recalled consumption of bean sprouts, six had eaten them in the home (four had purchased them from supermarkets). 10 had eaten them in take away meals or at restaurants. (One person had eaten them from both sources.)

In total, 94 of the 231 patient isolates underwent further typing and 87 of these were found to be of PFGE type SBARXB.0016.

Food investigation

The complex distribution network of bean sprouts and the results of routine microbiological testing of bean sprouts by suppliers for quality control purposes in the UK were also investigated. In August and September 2010, two UK suppliers identified in the investigation of the Scottish cases (suppliers A and B) had recorded the identification of a group C *Salmonella* in samples of bean sprouts which were intended to be cooked by consumers. None of these isolates were available for further characterisation. Routine regular testing of samples at an upstream supplier (supplier C, who supplies B, who in turn supplies A) had been consistently negative for many years.

Samples of bean sprouts from suppliers B and C were collected. *S. Bareilly* of a PFGE type indistinguishable from the outbreak cases was identified in a packet of bean sprouts produced by supplier C. Supplier C received mung bean seeds from upstream suppliers who sourced mung bean seeds from China or Myanmar. The investigation is ongoing.

Discussion

Our epidemiological and microbiological investigations implicated bean sprouts as a vehicle for *S. Bareilly* transmission, consistent with previous research showing that bean sprouts can be a vehicle for *Salmonella* transmission [1].

Case–control study designs are prone to a number of biases, the most important being recall bias. Although

there was a small amount of press interest in the possible link between *S. Bareilly* and bean sprouts at the time of the case–control study it is unlikely that many people in the general public were aware of a possible link between *Salmonella* and bean sprouts. In our study, controls were selected by random digit dialling, which may result in a low response rate and controls that are not representative of the general population. This is however unlikely to account for the strong observed association in our study.

Bean sprouts follow a complex path from farm to table that includes growing, harvesting, processing and shipping of mung bean seeds, followed by sprouting (normally at temperatures of 20–30 °C with high humidity) and distribution of the finished product. Seeds may arrive already contaminated or contamination may occur at any point of production and distribution. As in previous outbreaks, this investigation concluded that the seeds were likely to have been contaminated, as investigations at suppliers found little potential for cross contamination of sprouted seeds [7]. Based on the experience of this investigation, the methodology used for routine microbiological quality control testing of bean sprouts may not be sensitive to low levels of *Salmonella* contamination. This may have implications for future testing protocols.

The bean sprouts implicated in this investigation were not ready to eat products and would be safe to eat if the instructions for correct preparation (washing and cooking until piping hot) were followed. Public health interventions resulting from this investigation focussed on communications to the public and to public and environmental health professionals advising of the correct preparation of bean sprouts, and on improving food labelling where this was ambiguous. Given that *S. Bareilly* was only detected in raw mung bean sprouts intended to be cooked, rather than ready to eat bean sprouts, and the producers and suppliers have not been found to be at fault in our investigations, no product recalls were deemed necessary. No other interventions besides addressing the issue of potentially misleading labels were put in place with suppliers and producers. At the time of writing, the numbers of reports of *S. Bareilly* infection in the UK had fallen back to near expected levels.

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Control of a multi-hospital outbreak of KPC-producing *Klebsiella pneumoniae* type 2 in France, September to October 2009

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An outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* type 2 was detected in September 2009 in two hospitals in a suburb south of Paris, France. In total, 13 KPC-producing *K. pneumoniae* type 2 cases (four with infections and nine with digestive-tract colonisations) were identified, including a source case transferred from a Greek hospital. Of the 13 cases, seven were secondary cases associated with use of a contaminated duodenoscope used to examine the source case (attack rate: 41%) and five were secondary cases associated with patient-to-patient transmission in hospital. All isolated strains from the 13 patients: (i) exhibited resistance to all antibiotics except gentamicin and colistin, (ii) were more resistant to ertapenem (minimum inhibitory concentration (MIC) always greater than 4 mg/L) than to imipenem (MIC: 1–8 mg/L, depending on the isolate), (iii) carried the *bla*_{KPC-2} and *bla*_{SHV12} genes and (iv) had an indistinguishable pulsed-field gel electrophoresis (PFGE) pattern. These cases occurred in three hospitals: some were transferred to four other hospitals. Extended infection control measures implemented in the seven hospitals included: (i) limiting transfer of cases and contact patients to other wards, (ii) cohorting separately cases and contact patients, (iii) reinforcing hand hygiene and contact precautions and (iv) systematic screening of contact patients. Overall, 341 contact patients were screened. A year after the outbreak, no additional case has been identified in these seven hospitals. This outbreak emphasises the importance of rapid identification and notification of emerging highly resistant *K. pneumoniae* strains in order to implement reinforced control measures.

Introduction

The emergence of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* type 2 (hereafter referred to as KPC-2 *K. pneumoniae*) has been reported worldwide and is becoming a major clinical and public health concern [1]. Such pathogens remain rare in France although a few outbreaks have been reported [2]. In this paper we describe a multi-hospital outbreak of KPC-2 *K. pneumoniae*, which occurred in a suburb south of Paris, France, in September and October 2009.

Alert of a healthcare-associated infection

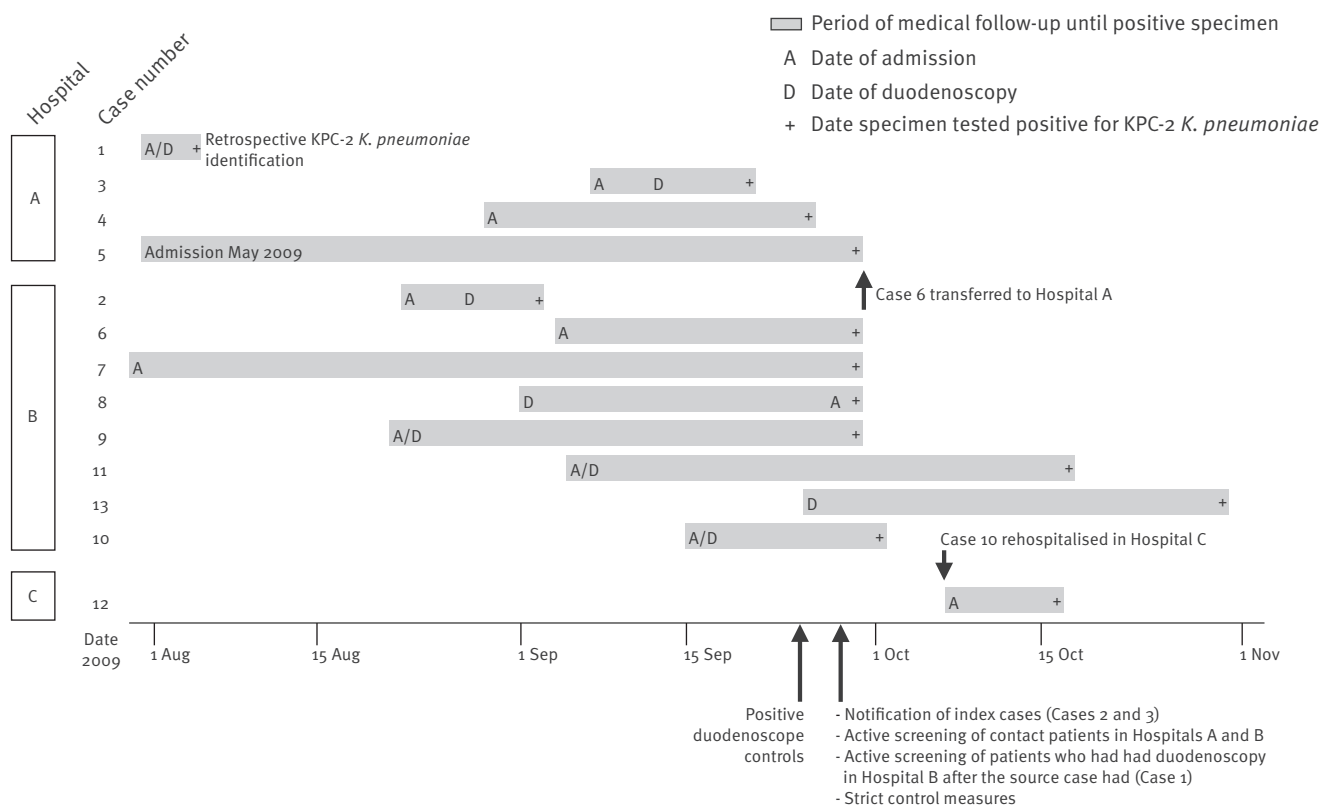
A national healthcare-associated infection early warning and response system was set up in France in 2001, coordinated at national level by the French public health surveillance institute (Institut de Veille Sanitaire, InVS) [3]. Through this system, two university hospitals (Hospitals A and B) in a suburb of south of Paris each reported KPC-2 *K. pneumoniae* bacteraemia in one patient at the end of September 2009 to the Regional Coordinating Centre for Nosocomial Infection Control (Centre de coordination de la lutte contre les infections nosocomiales (CCLIN Paris-Nord)) in northern France and to the regional health authorities. The case in Hospital A was notified the day before the notification of the case in Hospital B. These hospitals belonged to the same institution, the Assistance Publique–Hôpitaux de Paris.

Epidemiological investigations

In response to the notifications from the two hospitals, local infection control teams carried out epidemiological investigations with the support of the regional

FIGURE

Spatio-temporal description: multi-hospital outbreak of KPC-2 *Klebsiella pneumoniae*^a, France, September to October 2009 (n=13)



^a *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* type 2.

TABLE

Case description^a: multi-hospital outbreak of KPC-2 *Klebsiella pneumoniae*^b, France, September to October 2009 (n=13)

Case number	Hospital ^c	Duodenoscopy in Hospital B	Type of specimen tested	Infection/colonisation	Outcome as of 1 November 2010	Comment
1	A	Yes	Rectal swab	Colonisation	Alive	Source case (transferred from Greece)
2	B	Yes	Blood sample	Infection (bacteraemia)	Death unrelated to KPC-2 <i>K. pneumoniae</i>	Index case Hospital B
3	A	Yes	Blood sample	Infection (bacteraemia)	Death unrelated to KPC-2 <i>K. pneumoniae</i>	Index case Hospital A
4	A	No	Biliary fluid	Infection (biliary)	Alive	Contact of Case 3
5	A	No	Bronchial aspirate	Infection (pulmonary)	Death unrelated to KPC-2 <i>K. pneumoniae</i>	Contact of Case 3
6	B	No	Rectal swab	Colonisation	Alive	Contact of Case 2 Transferred to Hospital A
7	B	No	Rectal swab	Colonisation	Alive	Contact of Case 2
8	B	Yes	Rectal swab	Colonisation	Alive	–
9	B	Yes	Rectal swab	Colonisation	Alive	Transferred to Hospital D then to Hospital E
10	B	Yes	Rectal swab	Colonisation	Alive: urinary tract colonisation in 2010	Index case Hospital C Returned home from Hospital B, then re-hospitalised in Hospital C
11	B	Yes	Rectal swab	Colonisation	Death unrelated to KPC-2 <i>K. pneumoniae</i>	Underwent follow-up in Hospitals F and G
12	C	No	Rectal swab	Colonisation	Alive	Contact of Case 10
13	B	Yes	Rectal swab	Colonisation	Alive	Underwent follow-up in Hospital G

^a Reasons for hospitalisation included biliary or gall bladder lithiasis, gastrointestinal carcinoma, gastrointestinal haemorrhage, hepatic transplantation and peritonitis.

^b *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* type 2.

^c Hospital where KPC-2 *K. pneumoniae* was diagnosed.

infection control coordination centre and of the central infection control team of the Assistance Publique–Hôpitaux de Paris.

A case was defined as a person from whom KPC-2 *K. pneumoniae* was identified microbiologically in rectal swabs taken as part of systematic screening or in clinical specimens. A contact patient was defined as a person who shared the same unit and the same health-care workers as a case. All contact patients in hospital wards were listed and screening was proposed to them.

Outbreak description

Source case

Preliminary results of the epidemiological investigation at the end of September 2009 indicated that the first two cases notified from Hospitals A and B had undergone duodenoscopy in Hospital B at the end of August and in early September 2009 with the same duodenoscope. Following notification of these two cases, retrospective analysis of the charts of all patients who had undergone duodenoscopy since March 2009 (the date of the last maintenance by the manufacturer) in Hospital B with the same duodenoscope used for the two cases pointed to a likely source case – a patient examined by duodenoscopy at the end of July 2009 in Hospital B. The endoscopy was carried out in Hospital B, but the patient stayed in Hospital A, after being transferred from a hospital in Greece, where KPC-2 *K. pneumoniae* is endemic [4]. Screening for all *Enterobacteriaceae* resistant to third-generation cephalosporins, in a rectal swab on admission – routinely performed in Hospital A since a previous outbreak [2] – was negative for this likely source case, but a subsequent swab was positive (at the start of August 2009). The bacterial strain had first been considered as susceptible to carbapenems (with an imipenem minimum inhibitory concentration (MIC) of 1.5 mg/L), but during the outbreak investigation in September 2009, the laboratory of Hospital A detected the *bla*_{KPC-2} gene in this isolate. It was therefore retrospectively considered as the first isolate of KPC-2 *K. pneumoniae* in Hospital A, and the case from whom it was isolated as the source case of the outbreak.

Secondary cases

There were two further cases of KPC-2 *K. pneumoniae* infection (biliary and pulmonary) in Hospital A at the end of September 2009 (Cases 4 and 5); the infection was acquired by patient-to-patient transmission in the same ward. These patients had not undergone endoscopy but their stay overlapped with the stay in Hospital A of the first case notified by this hospital (Case 3) [5] (Figure).

Active screening of contact patients was then conducted in Hospitals A and B: all isolated extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* were screened for susceptibility to carbapenems (imipenem and ertapenem) and the MICs were determined.

No other cases were identified among 87 contact patients in Hospital A, but two new cases (Cases 6 and 7) were identified among 208 contact patients in Hospital B. A total of 295 of 342 contact patients (86%) were screened in these two hospitals.

Of the 17 patients who had undergone endoscopy in Hospital B since March 2009 with the same duodenoscope as that used for the source case, two were the notified index cases with bacteraemia, five had KPC-2 *K. pneumoniae*-positive rectal swabs, nine were negative after three screenings over the course of the two-week investigation and one died before the investigation started. The carrier status of one of these five KPC-2 *K. pneumoniae*-positive patients (Case 10) was determined at the start of October 2009, but the patient was discharged the same day, to go home. The patient was subsequently readmitted (in early October) to an intensive care unit in a third hospital (Hospital C), without information about their KPC-2 *K. pneumoniae* status. After the infection control practitioner of Hospital B had been informed by physicians about the patient's readmission to Hospital C, they informed their counterpart in Hospital C, so that screening and control measures could be implemented. As a result of screening of 25 contact patients in Hospital C, one secondary case was identified (Case 12).

Four cases were transferred to or had medical follow-up in four other hospitals (Hospitals D to G): one was in the same region as Hospitals A and B and three were in neighbouring regions. The cases' KPC-2 *K. pneumoniae* status was known at the time of transfer or follow-up. In Hospitals D and E, there were no contact patients; in Hospital F, three contact patients were screened and in Hospital G, 18 were screened (at least one rectal swab from each contact patient was obtained and screened). No further cases were identified in Hospitals D to G.

By the end of October 2009, 13 KPC-2 *K. pneumoniae* cases (four with infections and nine with digestive-tract colonisations) had been identified in Hospitals A to C (Table), comprising one source case, seven secondary cases among the 17 patients who underwent endoscopy with the same duodenoscope (attack rate: 41%) and five secondary cases among the 341 contact patients in Hospitals A to G (attack rate: 1.5%). There were no deaths related to KPC-2 *K. pneumoniae* infection. As of 1 November 2010, no new case involving the same strain has been identified in these seven hospitals.

Microbiological investigations

Carbapenem resistance of the isolated bacterial strains was initially detected by routine methods [6]. The MIC of imipenem and ertapenem was determined by Etest (Bio-Rad). The presence of the *bla*_{KPC-2} gene and *bla*_{SHV12} gene was identified by polymerase chain reaction (PCR) and DNA sequencing.

In Hospital A, all ESBL-producing *K. pneumoniae* strains that had been isolated in the six months before the outbreak were re-investigated, to screen for carbapenemase production using the modified Hodge test and ethylenediaminetetraacetic acid (EDTA)-disc synergy [7] and PCR.

Rectal swabs or clinical specimens were screened for *Enterobacteriaceae* resistant to third-generation cephalosporins by plating on Drigalski agar containing 0.5 mg/L cefotaxime and MacConkey agar containing 2 mg/L ceftazidime (AES Laboratoire) [5], a medium commonly used in France for ESBL-producing strains. Indeed strains resistant to carbapenems are also resistant to third-generation cephalosporins. All bacteria that grew were identified at the species level and tested for susceptibility to various drugs, including carbapenems. The laboratory of Hospital B performed pulsed-field gel electrophoresis (PFGE) of genomic DNA of the isolated bacteria using *Xba* 1 restriction enzyme.

Duodenoscope disinfection controls were performed according to the national recommendations of the French Ministry of Health published in 2007 [8]. After disinfection, 100 ml of sterile wash solution were aseptically injected in all channels of the endoscope. The wash solution was collected at the other end of the duodenoscope and cultured on standard culture medium at various temperatures. If the cultures were positive, each channel was tested individually. Bacterial colonies were counted and identified according to standard methods [6].

At the end of September 2009, despite disinfection of the endoscope, two microbiological controls of the duodenoscope used by the source and some subsequent cases revealed the presence of faecal flora (*Enterobacteriaceae* and *Enterococcus*), including KPC-2 *K. pneumoniae* strains (10^3 colony-forming units (CFU)/ml) with an indistinguishable PFGE pattern. No other multidrug-resistant bacteria were found.

As already described in reports focusing on microbiological aspects of this outbreak [5,9], the KPC-2 *K. pneumoniae* strains isolated from all 13 cases and from the duodenoscope showed resistance or intermediate susceptibility to all antibiotics except gentamicin and colistin. The MIC for imipenem varied between 1.5 mg/L and 8 mg/L, depending on the isolate. However, the MIC for ertapenem was always greater than 4 mg/L. PCR analysis of the outbreak strain identified the *bla*_{KPC-2} and the *bla*_{SHV-12} genes encoding respectively a carbapenemase and an ESBL [5]. Two other beta-lactamase genes were identified: *bla*_{TEM-1} and *bla*_{OXA-9}. Multilocus sequence typing, performed as previously described [10], showed that all strains belonged to sequence type (ST) 258, [11,12]. The PFGE patterns of all the isolates were indistinguishable.

Infection control measures

Evaluation of duodenoscope disinfection practices and maintenance

The duodenoscope in question had been acquired by Hospital B in 2003 and was therefore rather old in 2009, but had been regularly maintained. A new, automated cleaning device had been in use for a year; peracetic acid had been used instead of glutaraldehyde for disinfection. The cleaning and disinfection processes were consistent with guidelines [13] but the drying process was not optimal.

The duodenoscope was sent to the manufacturer during the outbreak investigation for maintenance, cleaning and disinfection. No signs of damage were identified. Disinfection procedures were reviewed and disinfection practices were observed by the local infection control team of Hospital B with the support of the regional infection control coordination centre and of the central infection control team of the Assistance Publique–Hôpitaux de Paris. The disinfection procedures were revised, to include a systematic and complete drying step after each disinfection cycle. After the outbreak, microbiological controls of the duodenoscope were performed more frequently (monthly). Since January 2010, controls have been carried out every three months (before the outbreak, they were performed every six months).

Hospital infection control procedures

Extended infection control measures were implemented in each of the seven hospitals involved by local infection control teams with the support of hospital administrators, coordinated by the regional infection control coordination centre and, in Hospitals A and B, also by the central infection control team of the Assistance Publique–Hôpitaux de Paris. The objective of these measures was to prevent future patient-to-patient transmission and included: (i) limiting transfer of cases and contact patients to other wards until the case was discharged from hospital, (ii) cohorting cases and contact patients in separate units with different healthcare workers, until discharge [14], (iii) flagging the presence of cases by displaying a specific poster or logo on the doors of cases' rooms and in the part of the ward where cases were cohorted, (iv) reinforcing hand hygiene (more and better use of hydroalcoholic solutions) and contact precautions, and (v) systematic screening of contact patients.

In Hospitals D and E, strengthened control measures were immediately implemented at the time of admission of cases, to ensure that no patients came into contact with a case.

Discussion

Carbapenemase-producing *K. pneumoniae* have increasingly been isolated from patients in healthcare settings worldwide [1] and are already endemic in some countries. In Europe, Greece has the highest prevalence of carbapenem-resistant *K. pneumoniae* strains;

other countries such as Israel and the east coast of the United States also have a high prevalence [15,16]. In France, a few KPC pneumoniae cases have been reported, mostly from patients transferred from hospitals in the three countries mentioned [1,17,18].

Hospital A receives numerous patients from hospitals in Greece and the Middle East and has already described an outbreak of Verona integron-encoded metallo-beta-lactamase (VIM)-producing *K. pneumoniae* and its control [14]. Stringent control measures – such as those described during the outbreak described in this report – are implemented on the admission of such patients, to prevent patient-to-patient transmission. In order to assist hospitals in the implementation of such control measures, national guidelines on screening patients transferred from abroad have been recently published in France [19].

The outbreak described in this report highlights the risk of transmitting multidrug-resistant bacteria through endoscopy, particularly through invasive procedures such as duodenoscopy, and by patient-to-patient transmission in hospital. Our analysis showed that seven of the 17 patients who underwent endoscopy with the same duodenoscope used for the source case were contaminated with the outbreak strain over a period of two months. Together with the indistinguishable PFGE pattern, this strongly suggests that this duodenoscope represented a persistent source of contamination. A review showed that endoscopy-associated outbreaks are related to inadequate endoscope cleaning, although the risk of exogenous infection from endoscopes that have been appropriately reprocessed is very low [20]. This review reinforces the need for: (i) adequate drying after each reprocessing cycle, (ii) reprocessing endoscopes after a period of non-use, (iii) microbiological surveillance and (iv) coordinated handling of post-contamination responses.

The outbreak presented here shows that it is possible to limit cross-transmission of multidrug-resistant bacteria by healthcare workers in a multi-hospital setting by implementing systematic investigation (including screening of contact patients) and extended control measures (including cohorting separately case and contact patients), as recommended by the French health authorities for controlling the spread of multidrug-resistant bacteria [19,21]. However, several weaknesses of the infection control organisation during this outbreak should be pointed out: (i) the source case was only identified retrospectively, due to the difficulty of identifying carbapenemase production in bacterial strains with a low level of resistance to some carbapenems; (ii) there was a delay in issuing an alert warning after the identification of the first two index cases in Hospitals A and B, which led to a high number of contact patients; (iii) one case from Hospital B was transferred to another hospital without information about the previous hospital stay of this patient or the patient's KPC-2 *K. pneumoniae* carrier status. The

lessons learnt from this outbreak may help to improve the efficiency of control in future outbreaks and to prevent further outbreaks.

Reactivity and preparedness of local and regional personnel (e.g. microbiologists, healthcare workers and infection control teams) are likely to be crucial in controlling emerging multidrug-resistant pathogens [22]. In addition, clear and comprehensive recommendations for microbiological laboratories will facilitate the early detection of carbapenemase-producing organisms [1]. This outbreak also demonstrates the usefulness of a coordinated healthcare-associated infection early warning and response system in rapidly implementing a multi-hospital investigation, providing assistance to hospitals for screening and infection control measures, and controlling the spread of an emerging pathogen.

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Clinical laboratory practices for detection and reporting of *Cryptosporidium* in community cases of diarrhoea in the United Kingdom, 2008

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To identify procedures employed by publicly funded clinical diagnostic laboratories in the United Kingdom (UK) for the detection of *Cryptosporidium* in community cases of diarrhoea, a telephone survey was conducted between August 2008 and January 2009 of all such laboratories that test stools from community-based patients. All 200 laboratories responded: 145 (72.5%) tested all stool samples for *Cryptosporidium*, while 55 (27.5%) applied selection criteria. There were country and regional differences in the proportion of laboratories selectively testing stools, which were significantly correlated with *Cryptosporidium* report rates to national surveillance (Spearman's rank correlation coefficient (r_s)=0.61, degrees of freedom (df)=11, $p=0.03$). Understanding of laboratory practice is fundamental to interpreting trends in surveillance data, estimating disease burden and identifying outbreaks, as well as providing important background information against which changes and effects of new public health regulations can be measured.

Introduction

Cryptosporidium is a protozoan parasite that infects a broad host range, causes gastrointestinal illness in humans and young animals and constitutes a significant risk to public health, especially among young children and immunocompromised patients with specific T-cell deficiencies [1]. Transmission is by the faecal-oral route. Particular challenges to control arise from the ability of the transmissive stage, the oocyst, to survive conventional water treatment and its resistance to chlorine disinfection, resulting in waterborne disease in developed and developing countries. Laboratory testing is necessary for differential diagnosis of gastroenteritis and implementation of appropriate control measures, and requires submission of a stool sample. However, clinicians and public health professionals should be aware that not all laboratories test all stools for *Cryptosporidium* and that *Cryptosporidium* testing is not necessarily specified in a test request for ova, cysts and parasites.

In the early 1990s, following a waterborne outbreak of cryptosporidiosis in Swindon, Wiltshire, and neighbouring parts of Oxfordshire, England [2], an expert group was established by the then Departments of Health and Environment to advise the United Kingdom (UK) Government on the significance of the presence of *Cryptosporidium* in water supplies. One of the recommendations of the ensuing report was that there should be a review and standardisation of clinical laboratory policies for examining faecal samples for *Cryptosporidium* [3]. Evidence from a two-year prospective study in 16 laboratories where all stools were tested for *Cryptosporidium* showed that 60% of positive stools were from children up to 15 years of age and that 90% were from people under 45 years of age [4]. Thus a joint working group recommended that, where possible, all specimens from symptomatic individuals should be tested, but where this was not feasible, children and adults up to 45 years should be tested [5]. As a minimum requirement, all children up to and including 15 years of age should be tested. Recommended methods were microscopic examination of faecal smears stained with auramine phenol (AP) or a modified Ziehl-Neelsen's (mZN) stain [6]. In the UK, stools are submitted fresh, without formalin, so concentration is not required routinely prior to staining. Where concentration is deemed necessary, modified methods should be used to minimise oocyst losses and prevent interference with the adhesion of oocysts to slides and with staining [6]. Examination of stained smears is required because the oocysts are too small (4–6 μm) for accurate identification by unstained observation of wet faecal films [6]. The national standard method of the Health Protection Agency (HPA) currently recommends that all samples from symptomatic individuals should be tested for *Cryptosporidium* [7] by microscopy using AP or mZN staining, with confirmation by staining a new smear using mZN stain [8]. The use of enzyme immunoassays (EIAs) is acknowledged but is not specified as a national standard method. Despite these recommendations, a variable pattern of testing has been identified previously [9–11]. In 2006 we sur-

veyed 169 laboratories in the UK: 36 (21.3%) applied selection criteria (unpublished data).

Cryptosporidium is included in Directive 2003/99/EC the European Parliament and the Council of the European Union [12], which directs monitoring and data collection of zoonoses and zoonotic agents. Cryptosporidiosis is therefore a notifiable disease within the European Union and laboratory-confirmed case data are collected through the European Surveillance System (TESSy). However, the disease is statutorily notifiable in only some European countries (e.g. Germany, Ireland and Sweden). In 2007, only 10 of the 30 countries in TESSy reported any cryptosporidiosis cases [13] and it is likely that there are substantial differences in ascertainment between countries [14]. Ireland reported the highest notification rates in 2007 (14 per 100,000 population), followed by the UK (six per 100,000 population). In the UK national surveillance has been based on voluntary, passive laboratory reporting of diagnosed cases (either by paper reports or through interconnected computer database modules to the health protection agencies). Prior to 2010 cryptosporidiosis was only statutorily notifiable as part of food poisoning notifications. However, under new regulations taking forward the modernisation of health protection law, the Health Protection (Notification) Regulations 2010, *Cryptosporidium* is included in the list of causative agents known as Schedule 2 and so detection of the parasite became notifiable by UK laboratories in October 2010, except in Scotland, where *Cryptosporidium* became notifiable on 1 January 2010 under the Public Health etc. (Scotland) Act 2008.

Baseline knowledge of the policies and practices for detecting and reporting *Cryptosporidium* is essential for assessing disease burden, comparing surveillance data, identifying outbreaks, and implementing and monitoring interventions. To establish clinical laboratory procedures prior to changes in notification requirements, and to identify changes since the previous surveys, we surveyed between August 2008 and January 2009 the policies of publicly funded laboratories throughout the UK concerning *Cryptosporidium* testing and reporting.

Methods

All 200 publicly funded clinical laboratories that test stools from community-based patients in the UK were contacted by telephone between 20 August 2008 and 27 January 2009. One person (B. Campbell) interviewed the consultant microbiologist, laboratory manager or lead biomedical scientist using a structured questionnaire. The questions were about selection criteria for testing stools for *Cryptosporidium*, diagnostic tests and reporting results, and referred to community cases of diarrhoea.

Data were recorded and analysed in Microsoft Excel. Regional and national numbers of reported cryptosporidiosis cases were obtained from the websites of the HPA (for England and Wales) [15], the Communicable Disease Surveillance Centre (Northern Ireland) [16] and Health Protection Scotland [17]. Report rates per 100,000 population were calculated using mid-year population estimates for 2008 [18] as denominator and were compared with the proportion of laboratories testing all stools. Spearman's rank correlation coefficient

TABLE

Laboratory testing of community cases of diarrhoea for *Cryptosporidium* and reports to national surveillance in the United Kingdom, 2008

Country or Government office region (England only)	Number of laboratories testing all stools for <i>Cryptosporidium</i> /number of laboratories (%)	Number of <i>Cryptosporidium</i> reports to national surveillance ^a (rate per 100,000 population)
England	100/154 (65)	3,885 (7.5)
East	12/18 (67)	469 (8.2)
East Midlands	7/8 (88)	547 (12.3)
London	7/23 (30)	197 (2.6)
North East	3/9 (33)	193 (7.5)
North West	24/27 (89)	673 (9.8)
South East	11/22 (50)	467 (5.6)
South West	10/15 (67)	458 (8.8)
West Midlands	14/17 (83)	383 (7.1)
Yorkshire and the Humber	12/15 (80)	498 (9.6)
Wales	14/14 (100)	239 (8.0)
Northern Ireland	5/6 (83)	119 (6.7)
Scotland	26/26 (100)	613 (11.9)
Total	145/200 (73)	4,856 (7.9)

^a Data sources: Health Protection Agency Centre for Infections (England and Wales) [15]; Public Health Agency Communicable Disease Surveillance Centre (Northern Ireland) [16] and Health Protection Scotland [17].

(r_s) was obtained by applying the formula for Pearson's r to the ranks of the two variables.

Results

Of the 200 laboratories surveyed, all confirmed that they tested specimens from community cases of diarrhoea. Of these, 154 laboratories were in England, 14 in Wales, 26 in Scotland, and six in Northern Ireland. A total of 145 (72.5%) tested all stool samples from community cases of diarrhoea for *Cryptosporidium* while 55 (27.5%) applied selection criteria. There were country and regional differences in the proportion of laboratories selectively testing stools: in Scotland and Wales all stools were tested, in Northern Ireland only one laboratory applied selection criteria, but in London, England, only seven of 23 laboratories tested all stools (Table).

There was a positive relationship between *Cryptosporidium* reports to national surveillance and completeness of laboratory testing at the regional level; the two were significantly correlated ($r_s=0.61$, degrees of freedom (df)=11, $p=0.03$) (Figure).

Of the 55 laboratories that selectively tested stools for *Cryptosporidium*, one or more of the following criteria were applied: age ($n=38$), immune status ($n=36$), stool consistency ($n=37$), duration of diarrhoea ($n=3$), overseas travel ($n=22$), farm visit or animal contact ($n=17$), clinician's request ($n=13$) and during an outbreak ($n=3$). The 38 laboratories that selected specimens according to age used the following categories: >6 months ($n=1$), 6 months to 60 years ($n=1$), <2 years ($n=1$), <5 years ($n=3$), <6 years ($n=1$), <8 years ($n=1$), <9 years ($n=1$), <10 years ($n=4$), <11 years ($n=1$), <12 years ($n=1$), <14 years ($n=2$), <15 years ($n=6$), <16 years ($n=9$), <45 years ($n=5$) and <50 years ($n=1$).

The diagnostic methods used were mainly staining of faecal smears, either by an mZN stain ($n=44$, 21.9% laboratories) or AP stain with a variety of counterstains

($n=151$, 75.5% laboratories). Of the laboratories using AP staining, 61 (40.4%) confirmed the finding by mZN staining. One laboratory also used immunofluorescence microscopy to resolve equivocal results. Parasite concentration methods were used prior to staining by seven laboratories; six used commercially available faecal parasite concentrators and one an in-house diethyl ether method. In the UK, stools are submitted fresh and so concentration is not usually required.

Five laboratories used commercially available EIAs as the primary test. Two of these were *Cryptosporidium*-only assays and three were combination assays for the simultaneous detection of *Cryptosporidium* and *Giardia*. All five laboratories using EIAs confirmed findings by a second method; the *Cryptosporidium*-only assays were confirmed by staining and microscopy and the combination assays with either staining and microscopy or commercially available *Cryptosporidium*-specific immunochromatographic tests.

Reporting for surveillance purposes was undertaken by 190 (95%) laboratories. Reporting to local authorities was also undertaken by 190 (95%) laboratories (not the same 190).

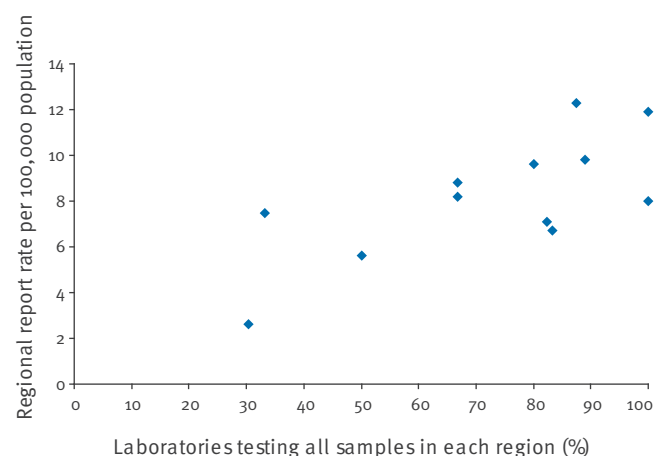
Discussion

Current UK guidance on laboratory testing is that stools from all community cases of diarrhoea should be tested for *Cryptosporidium* [7]. Although this was achieved by almost three quarters of laboratories nationally, there were large differences between the countries and regions and this may have an effect on the assessment of the burden of illness and ability to monitor outbreaks as well as measure changes in the number of reported cryptosporidiosis cases and report rates. It is of particular concern that 23 laboratories did not meet the minimum requirement of testing all children up to and including 15 years of age. Furthermore, where age criteria were applied these varied enormously with often no apparent rationale for the age cut-offs, leading to further inconsistencies in the data. The effect of age policy on case age distribution has been reported previously [11]. The value of testing adults, particularly up to age 45 years, has been demonstrated [4] and their inclusion in testing plays a key role in the ability to detect outbreaks, particularly those linked to drinking water, which affect all ages. It is well documented that one of the features of waterborne outbreaks is an excess of adult cases [19]. The use of inappropriate criteria has also been observed, especially submitted stool consistency, which is an unreliable predictor of *Cryptosporidium* positivity [20]. We discussed our findings with the regional microbiology network. The data demonstrate to clinicians that, even in the UK where testing is widespread, if cryptosporidiosis is suspected clinically then *Cryptosporidium* should be specified on the request form to ensure appropriate testing.

Despite some inadequacies, most stool samples are tested for *Cryptosporidium* in most regions of the UK

FIGURE

Relationship between regional rate of *Cryptosporidium* reports and completeness of laboratory testing in each region, United Kingdom, 2008



and this appears to be a relatively stable situation, with some notable exceptions. Overall, there has been little change between 2006 and 2008 with respect to the proportion of laboratories testing all samples. New developments in streamlining of laboratory testing are now available in the form of automated microscope slide staining and reliable alternatives to microscopy are provided by second-generation EIAs. These, particularly when coupled with automated processing and reading devices, simplify laboratory testing and assist in the standardisation of laboratory methods. Additional benefits have been demonstrated from the application of combination assays – for example, improved ascertainment of *Giardia*, particularly in groups not considered traditionally for testing [21]. Since our survey was completed, we are aware of at least two more laboratories that have replaced microscopy with EIA for *Cryptosporidium* and *Giardia* detection and more laboratories are using immunochromatographic assays. Alternatively, multiplex polymerase chain reaction (PCR) assays for routine diagnosis of various combinations of gastrointestinal pathogens including *Cryptosporidium* offer streamlined and modernised laboratory testing, and are routinely used in the Netherlands [22] and are under investigation in the UK.

Although an association cannot be proven by such analysis, there was a correlation between increased annual reporting rates and the proportion of laboratories testing all stools at the regional level. The analysis was imperfect because, while surveillance data may include reports from inpatients, only data related to testing community samples were used. However, we consider this would not lead to much discrepancy, as the vast majority of cases are diagnosed from community settings. Furthermore, there are other possible reasons for genuine regional or local variation in the numbers of reported cryptosporidiosis cases, such as exposure, environmental and socio-economic factors [23,24]. Despite this, the trend for correlation between regional reporting and testing rates is notable, as shown in the Figure. The higher cryptosporidiosis rates reported by the UK to TESSy, compared with most other European countries, are undoubtedly influenced in part by differences in approaches to laboratory testing and data collection. Standardised laboratory testing and reporting of pathogens in the European setting is important in minimising discrepancies, ensuring comparability of surveillance data and enabling rapid public health action in the event of international incidents and outbreaks [12].

A high proportion of laboratories already report cryptosporidiosis cases to local authorities for further investigation and to surveillance systems in the UK. Although statutory notification will ensure high ascertainment where all samples are tested for *Cryptosporidium*, the requirements of the new Health Protection (Notification) Regulations do not extend to mandatory testing, and there will still be an effect on the reported incidence of

cryptosporidiosis. This has been observed in Ireland, where *Cryptosporidium* became statutorily notifiable in 2004, but a large number of laboratories have opted to apply an age threshold as a selection criterion [25]. The interpretation of routine laboratory surveillance data is difficult without standardised policies, and without these, comparisons at regional, national and international levels are hampered [14]. Cryptosporidiosis remains an underreported disease despite the increase in testing in countries such as the UK. Understanding of laboratory practice is fundamental to understanding trends in surveillance data, estimating disease burden and identifying outbreaks.

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EMCDDA publishes guidelines on testing for HIV, viral hepatitis and other infections in injecting drug users

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On 1 December 2010, World AIDS Day, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) published guidelines on testing for HIV, viral hepatitis and other infectious diseases in injecting drug users (IDU) [1]. The new guidelines recommend a strategy to increase the uptake of testing for HIV and other infections among IDU in Europe and beyond. This would enable earlier treatment of infected individuals which lowers the risk of further spread.

Provided testing is carried out with informed consent, with pre- and post-testing counselling and the confidentiality of test results can be guaranteed, the guidelines recommend that IDU should be offered the following tests regularly (depending on infection risks this can be up to once or even twice per year):

- serology tests for HIV, hepatitis B, hepatitis C, hepatitis D (if there is evidence of chronic or recent hepatitis B), hepatitis A and syphilis;
- swab for culture from abscesses and skin lesions;
- biochemical tests (to determine levels of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and bilirubin);
- other general blood tests (to determine erythrocyte sedimentation rate (ESR), levels of C-reactive protein (CRP) and haemoglobin, and white blood cell count);
- tests for tuberculosis.

The uptake of testing for HIV and other infectious diseases among IDU is still often low in Europe (EMCDDA unpublished data). IDU remain a relatively `hidden` population, as they are often not regularly in contact with health services, and their infections may remain unnoticed for many years. Infectious diseases are among the most serious health consequences of injecting drug use and can lead to substantial health-care costs. IDU are vulnerable to a range of infectious diseases through a variety of risky behaviours, such as needle sharing and sex work, and because of circumstances such as poor hygiene, homelessness and poverty.

The guidelines are accompanied by a recommended package of prevention (including vaccination), primary care and referral routines in relation to IDU and infectious diseases. They were developed in collaboration with a range of experts and have been distributed to professionals throughout the European Union and worldwide.

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National Health Service 2010 Annual Evidence Update published on HIV and TB co-infection

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On 29 November 2010, timed to coincide with World AIDS Day 2010, the National Health Service (NHS) Evidence - *infections* launched its 2010 Annual Evidence Update covering human immunodeficiency virus (HIV) and tuberculosis (TB) co-infection, 'Seeing the whole picture' [1]. This aims to provide up-to-date evidence and expert commentaries related to the epidemiology and management of persons co-infected with HIV and TB.

In his commentary to the current update, Jeffrey Lazarus of the Global Fund to Fight AIDS, Tuberculosis and Malaria provides an overview of how HIV and TB infections interact and overlap and presents data underpinning his argument that TB incidence, to a large extent, is driven by the HIV epidemic.

The NHS spends several months preparing each annual update, carrying out searches to find the relevant evidence and finding experts to provide commentaries. The current update will run until 3 December 2010.

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