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Public health implications of an outbreak of rabies in arctic foxes and reindeer in the Svalbard archipelago, Norway, September 2011

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Between 16 September and 5 October 2011 rabies was diagnosed in two arctic foxes and eight reindeer in the Svalbard archipelago, in Norway. This outbreak occurs at the end of the reindeer hunting season and poses an increased risk to many people that were involved in the hunt. As of 28 September 2011, 280 people had received post-exposure prophylaxis. No human cases of rabies have occurred.

On 12 September 2011 an arctic fox (*Vulpes lagopus*) attacked a woman in Longyearbyen (Norway), a city with a population of approximately 2,000 inhabitants in the Svalbard archipelago in the Arctic. Later on the same day, a dog killed presumably the same fox. According to the tests performed by the Norwegian Veterinary Institute, this fox tested positive for rabies. In the two days following the event, the dog had contact with four people, including licking their hands and faces. On 18 September, two reindeer (*Rangifer tarandus platyrhynchus*) exhibiting unusual behaviour were found on the outskirts of Longyearbyen. Both were shot and, after testing by the Norwegian Veterinary Institute, were found to be positive for the rabies virus. The carcasses of three more reindeer found on 26 September more than 100 km south of Longyearbyen (Hornsund) have tested positive for rabies. Three additional reindeer and a second fox found between 25 September and 30 September on the outskirts of Longyearbyen also subsequently tested positive for rabies (Table 1).

Potential exposure of local population

The archipelago of Svalbard has a population of 2,539 with most people living in the city of Longyearbyen. Svalbard has a substantial multi-national population including 425 Russian and Ukrainian citizens living in the mining community of Barentsburg and many workers and researchers from other countries working in the archipelago. This outbreak occurs at the end of the reindeer hunting season in the Svalbard archipelago which lasts from 15 August to 20 September. Between 200 and 300 hunters participated in the hunt in which approximately 200 reindeer were killed. Although most participants are residents of Svalbard, visitors from outside the archipelago were also involved in the hunt. The Governor's office maintains a list of all participants. Groups of children from daycares and schools also participated in the hunt and in several cases were allowed to touch the animals after they were shot.

For hunting control and routine animal population surveillance purposes, hunters are asked to separate the mandible from the reindeer carcass and send it to the Svalbard Governor's office. This procedure may involve exposure to the animal's oral cavity, saliva and spinal

Table 1

Animals testing positive for rabies during the outbreak in the archipelago of Svalbard, Norway, as of 5 October 2011

Type of animal	Location	Date when the animal was found	Date of positive test results
Fox	Longyearbyen	12 Sep	16 Sep
Reindeer	Fuglefjellet[a]	18 Sep	21 Sep
Reindeer	Platåberget[a]	18 Sep	21 Sep
Reindeer	Platåberget[a]	25 Sep	28 Sep
Reindeer	Hornsund[b]	26 Sep	30 Sep
Reindeer	Hornsund[b]	26 Sep	30 Sep
Reindeer	Hornsund[b]	26 Sep	30 Sep
Fox	Bjørndalen[a]	28 Sep	3 Oct
Reindeer	Fuglefjellet[a]	30 Sep	5 Oct
Reindeer	Fuglefjellet[a]	30 Sep	5 Oct

[a] On the outskirts of Longyearbyen.
[b] More than 100 km south of Longyearbyen.

cord (while cutting off the head of the carcass). At the time the rabies virus was detected on 21 September in the first two reindeer, many people had already consumed cooked reindeer meat obtained during the hunting period and many still have meat in their freezers.

Rabies in the Arctic

Rabies is considered an endemic disease throughout most parts of the Arctic. Outbreaks of rabies have been previously reported in Canada, Russia and Greenland [1]. Rabies was first diagnosed in Svalbard in 1980 with an outbreak in the fox population [2]. From 1980 to 1999, a total of 25 animals were diagnosed with rabies on the islands of the archipelago, including three reindeer. While there is not a systematic surveillance system for rabies in place, there is significant concern among both officials and residents of Svalbard for the limited wildlife population. Animals exhibiting unusual behaviour or found dead for unknown reasons are routinely autopsied. In particular, all foxes found dead are autopsied. No further cases were reported until January 2011, when an arctic fox attacked a group of dogs at a meteorological station on Hopen, an island in the south-eastern part of the Svalbard archipelago [3]. Mainland Norway continues to be free of rabies. No human cases of rabies have occurred in Norway.

Transmission of rabies

The arctic fox is the main host of the virus, although how rabies is maintained in the relatively low-density fox population is unknown. Long incubation periods, prolonged periods of virus excretion and oral infection through frozen carcasses have been proposed as possible explanations [1]. The rabies virus is concentrated in the central nervous system (CNS) and is excreted through the salivary glands of rabid animals [4]. Animals can also excrete the virus through the saliva during a restricted period of time before any signs of disease occur [5]. The primary mode of transmission to humans is through the saliva in a bite from an infected animal. Rabies has also been transmitted, albeit rarely, via contamination of mucous membranes (i.e. eyes, nose, mouth), inhalation of virus-containing aerosol or via transplantation of an infected organ [6]. There may also be a limited risk that a dog that has bitten or eaten an animal infected with rabies may be able to temporarily retain the virus in its mouth and transmit it by biting or licking a person with scratches or wounds. The rabies virus is not transmitted through blood, faeces or urine. There is only negligible evidence that consumption of properly cooked meat from rabies-infected animals poses any risk for transmission of the disease and the World Health Organization reports that ingestion of raw meat is not a known source of human infection [7]. However, there may be limited risk of transmission associated with handling meat prior to cooking if one is in contact with the CNS, salivary glands or saliva, or if cross contamination from the CNS, salivary glands or saliva has occurred during the butchering process [8]. This is primarily a concern if the person handling the meat has cuts or scratches on the hands. While freezing does not kill the rabies virus, it is inactivated by exposure to sunlight and at temperatures above 56°C [9].

Post-exposure prophylaxis recommendations

Concern regarding the possible human exposure to rabies through the dog initiated a public health response by the hospital in Longyearbyen in conjunction with the Norwegian Institute of Public Health (NIPH), which was later expanded to include those involved in the reindeer hunt. At the time the rabies virus was detected in the two reindeer, many people were concerned because they had already consumed reindeer meat obtained during the hunting period and were unsure as to whether this posed a risk requiring post-exposure prophylaxis. The NIPH has issued recommendations on who should receive post-exposure prophylaxis based on different exposures, which are presented in Table 2.

The reindeer hunters who have been involved in removing the mandible of the reindeer (or have in other ways been in direct contact with saliva, the oral cavity or the central nervous system of a reindeer) fall under Group

Table 2

Post-exposure prophylaxis recommendations following the outbreak of rabies in the archipelago of Svalbard, Norway, September 2011

Exposure risk groups	Unvaccinated	Vaccinated
Group 1		
Individuals who have been bitten, scratched or cut through contact with animals suspected to be infected with rabies	Rabies immunoglobulin + 5 vaccine doses (Days 0, 3, 7, 14 and 30)	2 vaccine doses (Days 0 and 3)
Group 2		
Individuals who have been licked or bitten by a dog that has had direct contact within the previous two days with another animal suspected to be infected with rabies	5 vaccine doses (Days 0, 3, 7, 14 and 30)	2 vaccine doses (Days 0 and 3)
Group 3		
Individuals who have had direct contact with saliva, the oral cavity or the central nervous system of a reindeer (e.g. when removing the jaw of the animal)	5 vaccine doses (Days 0, 3, 7, 14 and 30)	2 vaccine doses (Days 0 and 3)

Source: Norwegian Institute of Public Health (Nasjonalt Folkehelseinstitutt).

3 and have been offered post-exposure prophylaxis. The Norwegian Food Safety Authority has, in collaboration with the NIPH and the Norwegian Veterinary Institute, issued advice that the consumption of well cooked reindeer meat does not present any health hazard with respect to rabies. The public is advised not to consume the brain or the spinal cord.

Since rabies has been observed on the islands for many years, vaccination of all dogs in the Svalbard archipelago was required before the outbreak. Dogs that have had contact with suspected rabid animals should be kept isolated in line with the World Organisation for Animal Health (OIE) recommendations and receive a booster vaccine. People who had been licked by the dog in the two days after it had killed the fox were recommended post-exposure prophylaxis.

Measures implemented

The Vaccine Department at NIPH has sent out approximately 1,100 doses of the rabies vaccine and 10 treatments of immunoglobulin against rabies in response to the Svalbard situation. These shipments have mainly been sent to the Svalbard archipelago although some have also been sent to parts of mainland Norway for people who have already left Svalbard. On Svalbard, vaccination is being organised by the local health authorities and administered at the hospital in Longyearbyen. Vaccination clinics are being simultaneously held at the school in Longyearbyen. The vaccine is also being made available in the settlement of New Ålesund, in the north-eastern part of the archipelago of Svalbard, in Barentsburg and to hunters located in other places on the island through helicopter transport. As of 28 September, only one person belonging to Group 1 has been identified and has received immunoglobulin in addition to vaccines as post-exposure prophylaxis. Four individuals belonging to Group 2 received post-exposure prophylaxis after having had contact with the dog that killed the rabid fox. People belonging to Group 3 (n=275) have received post-exposure prophylaxis; most of these individuals were involved in reindeer hunting. Although the Governor's office maintains a list of those involved in the hunt, most individuals have independently sought post-exposure prophylaxis. Despite the standing recommendation that hunters should be vaccinated against rabies, almost nobody receiving post-exposure prophylaxis had been previously vaccinated.

The dog that killed the rabid fox was vaccinated prior to the incident but has been placed in isolation for a period of 45 days in line with OIE recommendations as a precautionary measure. At present there are no indications that the dog may be infected with rabies.

In conjunction with the Norwegian Food Safety Authority, the Governor of Svalbard has urged the public to avoid contact with dead animals or animals exhibiting unusual behaviour.

Arctic foxes found in the city of Longyearbyen will be caught and killed by the authorities. Regular surveillance of the outskirts of Longyearbyen by helicopter for dead or sick animals is being performed. Fox traps have been set up in Longyearbyen as a measure to reduce the fox population in the area. In addition to being vaccinated against rabies, dogs must now be kept leashed or fenced in and under surveillance at all times. The public has been asked to notify the Governor's office of any animal carcasses or animals exhibiting unusual behaviour. Information is being provided to residents of Svalbard in Norwegian, English and Russian. Although the situation does not warrant travel advisories, for many years the NIPH has recommended that residents and visitors to the Svalbard archipelago likely to be in contact with wild animals (such as hunters, scientists and wildlife explorer) should receive the rabies vaccine.

Conclusion

Reindeer hunting is a popular activity that annually involves up to 300 people, including children. The rabies outbreak in the Svalbard archipelago has demonstrated that people engaging in activities involving contact with wild animals in rabies-endemic areas should consider being vaccinated. The recent events have had a significant public health impact on the residents of Svalbard, in particular for those involved in reindeer hunting.

Table 3

Post-exposure prophylaxis given following the outbreak of rabies in the archipelago of Svalbard, Norway, as of 28 September 2011 (n=280)

Exposure risk groups[a]	Number of people who received post-exposure prophylaxis[a]
Group 1	1
Group 2	4
Group 3	275
Total	**280**

[a] As described in Table 2.

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Suspected transmission of tuberculosis in a maternity ward from a smear-positive nurse: preliminary results of clinical evaluations and testing of neonates potentially exposed, Rome, Italy, 1 January to 28 July 2011

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We report preventive measures adopted after tuberculosis (TB) transmission from a nurse to a newborn assessed in late July 2011. All exposed neonates born between January and July 2011 were clinically evaluated and tested by QuantiFERON TB gold in-tube; newborns testing positive were referred for prophylaxis. Of 1,340 newborns, 118 (9%) tested positive and no other active cases of TB were found. Active surveillance for TB will be continued over the next three years for all those exposed.

Case descriptions

Case one

On 18 July 2011, a case of pulmonary and extra-pulmonary (splenic) tuberculosis (TB) was diagnosed in a four-month old infant at the children's hospital 'Bambino Gesù' in Rome, Italy. The diagnosis was confirmed by microscopic and sputum culture. According to international guidelines for TB prevention [1], all household members (parents and siblings, and two close contacts who took care of the baby) were screened for TB. No active TB cases were found and only the mother had a positive tuberculin skin test (TST) but was QuantiFERON TB gold in-tube (QFT-IT) TB-negative, most likely due to Bacillus Calmette-Guérin (BCG)-vaccination as a child. Although they tested negative, the two siblings, both below five years of age, were referred for prophylaxis.

Case two

On 26 July, pulmonary TB was clinically diagnosed in a patient at 'Gemelli' hospital, Rome and hereafter microbiologically confirmed by sputum culture as *Mycobacterium tuberculosis*, sensitive to first-line drugs, at the National Institute for Infectious Diseases L. Spallanzani, Rome. The patient, a nurse by profession, reported onset of night cough since the beginning of April 2011 and a mild asthenia that was attributed to the daily work and diet, as well as loss of 17 kg body weight (corresponding to almost 20% of the total weight). The loss of weight was interpreted as intentional since she had been on a low-calorie diet since February 2011. In June 2011, a sinusitis had been diagnosed and beclometasone aerosol prescribed.

As soon as the nurse was suspected as a case of TB, investigation of household members found her husband and son TST-positive. Furthermore, the husband had a history of exudative pleurisy in 2004. Pleural fluid and sputa had resulted negative for *M. tuberculosis* isolation by culture at that time; the husband had nevertheless undergone a full course of TB therapy, consisting of two months of quadruple therapy with isoniazid, rifampicin, ethambutol and pyrazinamide, followed by four month of double therapy with isoniazid and rifampicin with a supplementation of vitamin B over the whole six months. Subsequently, he had successfully recovered from lung lesions, as assessed by radiology, and in terms of clinical symptoms. Therefore he had been diagnosed with pleural TB based on clinical grounds. At that time, the nurse was not tested by TST since her husband was deemed not contagious.

Because the nurse had been working in the maternity ward, at the same university hospital where the child (case one) was born in March 2011, an epidemiological link between the two cases was suspected. DNA fingerprinting of the isolated and cultured samples of *M. tuberculosis* in the two cases confirmed the link.

Epidemiological background

Italy is a low TB prevalence country according to the World Health Organization (WHO) definition [2,3]. The annual incidence in Lazio (the region including Rome, 5.6 million inhabitants), taking into account under-reporting, has been estimated to be around 11/100,000 [4]. Only 30 cases in children under one year of age have been reported annually to the local health authorities and regional public health agency in the last five years (12/100,000) [5].

Very few nosocomial outbreaks involving newborns have been described to date [6-14]. Here we report a case of TB that occurred in a four-month old infant potentially linked to a nurse diagnosed with TB who worked in the maternity ward when the child was born.

Investigation of potentially exposed newborns

Immediately after diagnosis of pulmonary TB in the nurse working in the maternity ward, a task force, including public health authorities, hospital infection control authority, and experts from the paediatric and infectious disease reference centres, defined the protocol described below for epidemiological investigation and preventive treatment.

The exposed group was defined as all the newborns (n=1,738) who were cared for in the maternity ward from three months before symptoms onset in the nurse to two days after the nurse's last working day (i.e. from 1 January to 28 July, 2011). Parents were contacted by phone and by mail, and counselled about the potential risk of infection and invited for a clinical visit and a screening test for the child in three paediatric outpatient clinics located in three different hospitals in Rome.

The task force decided to use QFT-IT (Cellestis Limited, Carnegie, Victoria, Australia) to screen newborns for several reasons: first, previous outbreak investigations of maternal wards suggested a lower sensitivity to latent TB infection of TST in neonates than in adolescents or adults [6-8,15]; second, it has been suggested to be more accurate than TST in low- and middle-income countries on children [16]; and, third it does not require, as does the TST, a second visit to assess results, which may be problematic for such a large population. However, there are very few studies of QFT-IT accuracy in newborns [16,17]. In any case, all children were tested by QFT-IT from 18 August. Since birth and potential exposure to *M. tuberculosis* were concurrent, and exposure was limited to one to two days during the hospitalisation in most cases, in the neonates below 12 weeks of age tested at the screening in August, a second QFT-IT and clinical evaluation were planned at the completion of three months of age. This is in agreement with guidelines that indicate an evaluation of contacts after the completion of three months from exposure.

The cut-off value for a positive test was 0.35 International Units(IU)/mL of interferon (IFN)-gamma in the sample after stimulation with the specific antigens, as suggested by the manufacturer. The task force decided to refer only those children positive to QFT-IT for isoniazid prophylaxis, since the exposed cohort had a priori a quite low probability of infection. Protocol dictated that children with a positive QFT-IT were referred for a chest X-ray and an additional clinical visit. An educational programme for the paediatricians of the exposed children, covering the clinical aspects of TB in that age group, was planned.

Tests whose results are reported here, were all performed from 18 August to 10 September, 2011. The table shows preliminary results of 1,340 tests for neonates whose parent or guardian consented to testing and examination. More potentially exposed neonates were tested after 10 September but the results are not yet known. Up to 10 September, a total of 118 (9%) positive QFT-ITs were obtained and only three (0.2%) indeterminate (low Mitogen Response, i.e. <0.5 IU/mL). The three children were retested after a month, after which they were responsive to the mitogen, and negative to QFT-IT. There was no clear trend from exposure (birth) month. Due to the immaturity of immune system, it was surprising to find that neonates scored QFT-IT positive under five weeks of age (11%, 9/79).

It is of note that, although not specified in the protocol, most QFT-IT positive infants subsequently were tested also with the TST, to check if the TST would confirm the positive QFT-IT. The infant diagnosed with active TB was both QFT-IT- and TST-positive, while the children

Table

Results of QuantiFERON TB gold by month of birth in the cohort of children potentially exposed to a nurse with active tuberculosis, Rome, Italy, January–July 2011 (n=1,340)

Month of birth in 2011	QuantiFERON TB gold	
	Total tested	Positive
	N[a]	N (%)
January	122	15 (12)
February	170	20 (12)
March	209	21 (10)[b]
April	214	13 (6)
May	224	15 (7)
June	223	15 (7)
July	174	19 (11)
Unknown	4	0 (0)
Total	**1,340**	**118 (9)**

a Three childrens' samples with low Mitogen Response (i.e. <0.5 IU/mL) were considered indeterminate; after a month these children were retested after which they were mitogen-responsive and QuantiFERON in-tube negative. For 15 cases, negative to gamma-interferon, Mitogen was not performed and for one case the result was missing.

b Table does not include the only confirmed case of active tuberculosis that occurred in an infant born in March.

without active TB, who had a QFT-IT positive result, were TST negative. To date, no other person with active TB has been identified.

Unfortunately there are no estimates of QFT-IT sensitivity and specificity in newborns [16-20]. Therefore, to estimate the proportion of true and false positives, QFT-IT test results from a sample of unexposed children of the same age are needed, but ethical and practical issues make this problematic. On the other hand, the sensitivity of immune-based tests is known to be low, in particular in the first weeks after infection and birth. Furthermore, it is possible that some infected children test false negative to QFT-IT. For these cases an active surveillance of symptoms by parents and paediatricians has been planned.

In conclusion, in this investigation 118 of 1,340 (9%) newborns who had been exposed to a nurse with active TB were found to have a positive QFT-IT. The majority of the neonates with a positive QFT-IT were tested also by TST and found to be TST-negative. After two to nine months of follow-up, none of the QFT-IT-positive neonates progressed to active TB, indicating a low predictive value of this assay for progression to active TB in this population. The only case of active TB found in this cohort was positive, at the time of TB diagnosis, to both TST and QFT-IT. This cohort will be carefully followed for the next three years.

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Cluster of travel-associated Legionnaires' disease in Lazise, Italy, July to August 2011

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Since 18 August 2011, 17 cases of travel-associated Legionnaires' disease have been reported. They were tourists from five European countries who had stayed in five accommodation sites in Lazise, Italy. The dates of symptom onset ranged from 18 July to 25 August 2011. Control measures were implemented and no further cases associated with stays at the sites have been reported after disinfection. Timely notification of any further cases potentially associated with stay in Lazise is recommended.

Cluster description

A total of 17 cases of travel-associated Legionnaires' disease have been reported since 18 August 2011 that were associated with a stay in several accommodation sites in Lazise, Italy. All cases – seven from the Netherlands, six from Germany, two from Italy, one from Austria and one from Denmark – stayed at five different accommodation sites (two campsites and three hotels) in Lazise between the beginning of July and end of August 2011. Dates of symptom onset ranged from 18 July 2011 to 25 August 2001 (Figure). The ages of the cases ranged from 42 to 78 years (mean: 57; standard deviation: 11.9) and the male to female ratio was 3.3 to 1.

Background

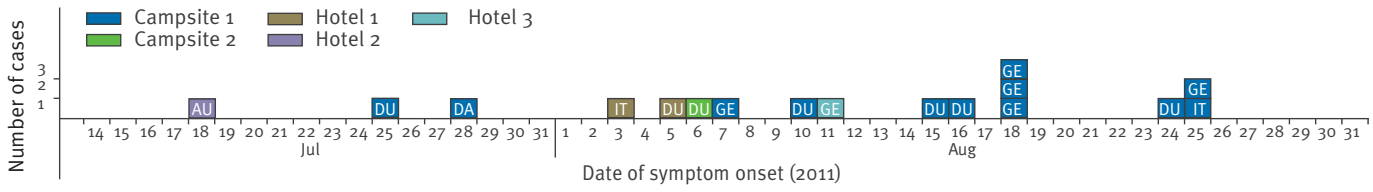
Legionnaires' disease is a lung infection caused by *Legionella* bacteria. The bacteria live in water or wet soil and must be inhaled to cause infection. *Legionella* can cause a severe form of pneumonia (Legionnaires' disease), which in Europe can be fatal for about 5–15% of people with the disease, but it can also cause a mild influenza-like infection without pneumonia, called Pontiac fever [1].

Over the last 10 years, the number of cases of Legionnaires' disease in Italy has been steadily increasing, from 325 cases in 2001 to 1,200 cases in 2009, with an incidence in 2009 of 2 per 100,000 population [2,3]. The number of cases of travel-associated Legionnaires' disease has also been increasing: every year, several clusters associated with accommodation sites, involving tourists from Italy and elsewhere in Europe, are reported [4,6]. Most of this increase has been attributed to improved diagnostic tools, in particular the urinary antigen detection test [7].

The European Legionnaires' Disease Surveillance Network (ELDSNet), coordinated by the European Centre for Disease Prevention and Control (ECDC) since

Figure

Cases of travel-associated Legionnaires' disease by date of symptom onset and nationality, Lazise, Italy, notified by ELDSNet as of 21 September 2011 (n=17)



AU: Austrian; DA: Danish; DU: Dutch; GE: German; IT: Italian.
ELDSNet: European Legionnaires' Disease Surveillance Network.

April 2010, carries out surveillance of Legionnaires' disease, involving all European Union Member States, Iceland and Norway. It aims to identify relevant public health risks, enhance disease prevention and monitor epidemiological trends. In this context, surveillance of travel-associated disease is carried out on a day-to-day basis to inform urgent public health action, with the aim of preventing subsequent cases. Each travel-associated case of Legionnaires' disease diagnosed in a participating European country is reported by national ELDSNet collaborators to ELDSNet as quickly as possible. If other cases are found to have been associated with a particular accommodation site within a two–year period, a cluster is identified. A rapid risk assessment of the accommodation site associated with the cluster is undertaken by the country in which the site is located: the results are reported to ECDC and shared with all countries in the network [8,9].

Testing isolates and data collection

Of the 17 reported cases reported in Lazise, 16 were confirmed by a urinary antigen test and one case remained probable because diagnosis was on the basis of a single high *Legionella*-specific antibody titre. *Legionella pneumophila* serogroup 1 was isolated from two patients: one had stayed at Campsite 1 and one at Campsite 2. There were no deaths.

Lazise is a small town located about 20 km north-west of Verona, by Lake Garda (the largest lake in the country). It has 7,000 inhabitants and there are an estimated 60,000 visitors during the summer holiday period. Legionnaires' disease was not reported in Lazise inhabitants in July and August 2011. The disease has been reported in tourists staying in neighbouring villages in the Lake Garda area, as expected based on the previous years' notifications (unpublished data).

Patients were contacted by ELDSNet national collaborators in their country of residence. Information about potential exposure in the 10 days preceding the onset of symptoms (incubation period for Legionnaires' disease is 2–10 days) was obtained using a standardised questionnaire: national ELDSNet collaborators of the countries where cases were reported recorded the details in an ad hoc restricted-access web-based database set up by ELDSNet. Analysis of the data revealed common accommodation sites but no other common exposure.

Ongoing investigations

Epidemiological and environmental investigations, which started immediately after notification of the cluster by ELDSNet on 19 August 2011, are ongoing. The Istituto Superiore di Sanità is supporting the local health authorities in Lazise.

Of the 17 reported cases, 12 had stayed in Campsite 1 (accommodating about 3,500 people), two had stayed in Hotel 1 (with about 40 rooms), two in two different hotels (Hotels 2 and 3 with about 50 rooms each) and one in Campsite 2 (accommodating about 1,800 people).

Three of the five accommodations sites (Campsite1, Campsite 2 and Hotel 1) were found to be within approximately 500 metres of each other. The water sources for the five accommodation sites are different: the two campsites are supplied by private wells while the three hotels are supplied by the same public service. Local rapid risk assessment was promptly carried out [10] and several water samples were collected for testing by the regional and the national reference laboratories according to procedures indicated for the control and prevention of legionellosis [11]. In the first round of sampling, 56 samples of cold and hot water were collected from water tanks, taps, shower heads, swimming pools, water sprinklers, decorative fountains and jacuzzis at the five accommodation sites. Two samples from Campsite 1 were found positive for *L. pneumophila* serogroup 1, with a concentration of 900 and 4,100 colony forming units per liter (CFU/L). These two samples had been collected from distal water outlets in one of the seven washing and toilet facilities. In Hotel 2, three samples were found positive for *L. pneumophila* serogroup 1, at concentrations ranging from 2,000 to 12,000 CFU/L . *L. pneumophila* serogroup 2-14 was isolated from other water points in all five accommodation sites.

No cooling towers were found in Lazise and its outskirts. To date, no installations have been identified as a potential source of *Legionella*.

Typing of *Legionella* isolates

The two *L. pneumophila* serogroup 1 clinical isolates were characterised by sequence-based typing [12]: both were sequence type (ST) 23, as were the two *L. pneumophila* serogroup 1 isolates from the environmental samples. Further molecular investigations are ongoing.

Control measures

A rapid risk assessment conducted promptly in all five accommodation sites allowed us to implement control measures. Disinfection of the water systems in all five accommodation sites involved was carried out as a control measure and all devices generating aerosols (e.g. spa pools, lawn sprinklers and decorative fountains) were immediately deactivated. Hospitals and general practitioners (GPs) in the area were alerted in order to enhance clinical surveillance of the disease. People staying at Campsite 1 (which reported the greatest number of cases) and for whom email addresses were available were informed by email of the ongoing cluster of the disease and were encouraged to contact their GPs if they developed symptoms. Managers of all the accommodation sites, spas and other recreational sites in the municipality were also informed through a note issued by the Mayor of Lazise and were made aware of the importance of adopting adequate measures to prevent legionellosis.

Environmental sampling, repeated after disinfection of the water systems, was negative for *Legionella* and no further cases have been notified after the risk management measures were adopted.

Conclusion

As a common source of infection in Lazise has not yet been identified, there may be an ongoing risk of exposure to *Legionella* for persons visiting or residing in the town. For this reason, we encourage timely notification of further cases potentially associated with stay in Lazise.

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Trends in yearly prevalence of third-generation cephalosporin and fluoroquinolone resistant *Enterobacteriaceae* infections and antimicrobial use in Spanish hospitals, Spain, 1999 to 2010

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Escherichia coli, *Klebsiella pneumoniae*, and *Enterobacter* spp. are a major cause of infections in hospitalised patients. The aim of our study was to evaluate rates and trends of resistance to third-generation cephalosporins and fluoroquinolones in infected patients, the trends in use for these antimicrobials, and to assess the potential correlation between both trends. The database of national point prevalence study series of infections and antimicrobial use among patients hospitalised in Spain over the period from 1999 to 2010 was analysed. On average 265 hospitals and 60,000 patients were surveyed per year yielding a total of 19,801 *E. coli*, 3,004 *K. pneumoniae* and 3,205 *Enterobacter* isolates. During the twelve years period, we observed significant increases for the use of fluoroquinolones (5.8%–10.2%, p<0.001), but not for third-generation cephalosporins (6.4%–5.9%, p=NS). Resistance to third-generation cephalosporins increased significantly for *E. coli* (5%–15%, p<0.01) and for *K. pneumoniae* infections (4%–21%, p<0.01) but not for *Enterobacter* spp. (24%). Resistance to fluoroquinolones increased significantly for *E. coli* (16%–30%, p<0.01), for *K. pneumoniae* (5%–22%, p<0.01), and for *Enterobacter* spp. (6%–15%, p<0.01). We found strong correlations between the rate of fluoroquinolone use and the resistance to fluoroquinolones, third-generation cephalosporins, or co-resistance to both, for *E. coli* (R=0.97, p<0.01, R=0.94, p<0.01, and R=0.96, p<0.01, respectively), and for *K. pneumoniae* (R=0.92, p<0.01, R=0.91, p<0.01, and R=0.92, p<0.01, respectively). No correlation could be found between the use of third-generation cephalosporins and resistance to any of the latter antimicrobials. No significant correlations could be found for *Enterobacter* spp.. Knowledge of the trends in antimicrobial resistance and use of antimicrobials in the hospitalised population at the national level can help to develop prevention strategies.

Introduction

Enterobacteriaceae are a major cause of infections in hospitalised patients [1]. Among them, the most frequent are *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. [2], with *E. coli* being the most frequent cause of bacteraemia, and community and hospital-acquired urinary tract infections (UTI) [3]. Bacteria of the genus *Klebsiella* frequently colonise the gastrointestinal tract, but can also be associated with opportunistic infections, including those of the urinary and respiratory tract. These organisms can spread rapidly among patients, mostly via the hands of hospital personnel, causing nosocomial outbreaks [4]. Within the *Klebsiella* genus, *K. pneumoniae* is the second leading cause of gram-negative bloodstream infections after *E. coli* [3]. In the family of the *Enterobacteriaceae*, *Enterobacter* spp. are also frequent pathogens that can cause opportunistic infections in hospitalised patients, especially in those who are immunocompromised or have mechanical ventilation support [1].

Fluoroquinolones and third-generation cephalosporins are classes of antibiotics frequently used for the treatment of infections caused by all of these organisms [1]. As a result of their extensive use, a continuous decline of the therapeutic effectiveness of these antimicrobial agents has been observed. This was predicted and seems unavoidable [5].

The aim of our study was to evaluate the rates and trends of resistance to different antimicrobials, particularly fluoroquinolones and third-generation cephalosporins, in the main *Enterobacteriaceae* causing nosocomial and community-acquired infections in hospitalised patients in Spain over a twelve year period (1999–2010), the trends in use of these antimicrobials within this period, and to assess a potential correlation between both trends.

Methods

Since 1990, point prevalence study series of nosocomial and community-acquired infections among patients hospitalised in acute care facilities have been conducted in Spain (Estudio de prevalencia de las infecciones nosocomiales en los hospitales españoles – EPINE study). Each year in May, acute care hospitals in Spain are requested to voluntarily join the EPINE prevalence study. In some of the regions it is compulsory to conduct this study or any other prevalence audit. Participating hospitals fill a standardised questionnaire on each hospitalised patient as well as provide overall data on the hospital and the hospital's wards. Every participating hospital designates a hospital contact point as the person responsible for the survey at the hospital level and for communicating with the EPINE executive board.

Infection diagnosis relied on Centers for Disease Control and Prevention (CDC) case-definitions for nosocomial infections [6]. Infections that met the CDC criteria but developed outside of the hospital or in the first 48 hours upon admission were categorised as community-acquired infections. In addition to information on the source of the infection and the associated microorganisms, the patient forms collected from the hospitals included demographic data (age and sex), use of antimicrobial (as the number of patients receiving any antibiotic per 100 patients on the day of the survey), type of ward (general medical as opposed to a surgical, intensive care, paediatric or obstetric ward), and size of the hospital as measured by the number of beds (small: less than 200 beds, medium: 200–500 beds, large: more than 500 beds). The survey was performed by trained doctors, nurses and, in some hospitals, medical students. All the forms were revised for inconsistency and a percentage of them (10–15%) were validated by the hospital contact point after revising medical records. Hospital validated forms were sent to an independent central analysis unit. A hospital report was sent back to every participating hospital to avoid possible disagreements before final integration of the collected results in a centralised database. We focused our analysis on resistance and antimicrobial use during the period from 1999 to 2010.

Standard microbiological procedures were used by different institutions in their corresponding microbiology departments or laboratories that followed accreditation or certification procedures following local health authorities' requirements. Interpretive criteria (breakpoints) for susceptible, intermediate and resistant categories were those included in the Clinical and Laboratory Standards Institute guidelines [7,8]. Resistant isolates included both intermediate and resistant isolates as reported by the microbiological laboratory to the clinician. As not all the microbiology laboratories tested the same antibiotics in susceptibility testing, resistance rates were expressed as percentage of isolates that were resistant to fluoroquinolones (ciprofloxacin or levofloxacin) or third-generation cephalosporins (ceftriaxone, cefotaxime or ceftazidime). No further characterisation of resistance mechanisms was recorded. Comparisons of characteristics of infections were made by Pearson's chi-square test. To determine significant trends over time of resistance rates and proportion of use, the Cochran-Armitage test was used. For correlation of antimicrobial use and the annual prevalence rates, Spearman correlation coefficient and regression coefficient were calculated. A p value <0.05 was considered significant. All calculations were performed with Stata/SE 9.0 statistical software.

Results

Between 1999 and 2010 an average of 265 hospitals per year (increasing from 233 in 1999 to 287 in 2010) participated in the EPINE survey yielding a sample of almost 60,000 hospitalised patients per year. Of a total of 303 hospitals participating in the survey at any given year, 230 took part in the entire twelve-year series.

During the period from 1999 to 2010 a total of 19,801 *E. coli*, 3,004 *K. pneumoniae* and 3,205 *Enterobacter* spp. isolates — including mainly *E. cloacae* (72%) and *E. aerogenes* (18%) species — causing nosocomial or community-acquired infections were recorded. The main characteristics of the corresponding infections for the period from 1999 to 2010 are displayed in Table 1. More than half of the infections occurred in patients aged over 65 years. *E. coli* infections were slightly more frequent in females, in contrast with *K. pneumoniae* or *Enterobacter* spp. infections (p values<0.001). Overall, about three quarters of infections were identified in patients hospitalised in medical or surgical wards but for *Enterobacter* spp. infections the ratio medical/surgical ward was inversely (0.64) related to the ratio for *E. coli* or *K. pneumoniae* (1.42 and 1.56, respectively). Intensive care unit (ICU) infections accounted for 14% and 17% of *K. pneumoniae* and *Enterobacter* spp. infections respectively, in contrast to only 7% of *E. coli* infections. UTI was the most common localisation of infection for *E. coli* and for *K. pneumoniae* but not for

Enterobacter spp.. Bloodstream infection represented 11.3%, 11.4% and 14% of the infection locations for each microorganism.

Escherichia coli *infections*

The characteristics of patients and hospitals where third-generation cephalosoporin resistance was found are shown in Table 2. Rates of resistance by type of ward ranged from 4.4% in gynecology and obstetrics to 13.3% in ICU patients, and by localisation of infection from 8.7% for UTI to 14.7% for respiratory infections. Information on fluoroquinolone resistance can also be found in table 2. Fluoroquinolone resistant *E. coli* infections were detected in patients from both sexes and all age groups seemed to be affected (although there appeared to be lower rates in age groups younger than 16 years old). Fluoroquinolone resistant *E. coli* could be community-acquired, but was more frequent in nosocomial infections, and in the largest hospitals. There were differences in the rates of fluoroquinolone resistance related to the type of ward and site of infection ranging from 5.4% in paediatric to 31.4% in medical wards and 32.6% in other hospital wards, and from 22.9% for surgical site wounds to 30.9% for respiratory tract infections (Table 2).

Klebsiella pneumoniae *infections*

Nosocomial infections as well as hospital size and type of ward, were associated with third-generation cephalosporin resistance. No difference for fluoroquinolone resistance was found by sex of the patients. High rates of fluoroquinolone resistant *K. pneumoniae* were found

Table 1

Main characteristics of patients infected by *Escherichia coli, Klebsiella pneumoniae,* or *Enterobacter* species in Spanish hospitals, Spain, 1999–2010 (n=672,362)

	Escherichia coli		*Klebsiella pneumoniae*		*Enterobacter* spp. *(Enterobacter cloacae)*[a]	
	n	%	n	%	n	%
Sex						
Male	9,004	46.2	1,635	55.3	2,048 (1,432)	64.8 (62.9)
Female	10,498	53.8	1,321	44.5	1,114 (843)	35.2 (37.1)
Age (in years)						
≤1	1,165	5.9	178	5.9	178 (137)	5.6 (6.0)
1–15	567	2.9	40	1.3	48 (38)	1.5 (1.7)
16–45	2,116	10.7	399	13.3	470 (322)	14.7 (14.0)
46–65	3,963	20	724	24.2	802 (570)	25.1 (24.8)
>65	11,964	60.5	1,654	55.2	1,696 (1,233)	53.1 (53.6)
Localisation of infection						
Urinary tract	10,051	51.2	1,056	35.6	494 (321)	15.6 (14.1)
Surgical site	2,908	14.8	384	12.9	876 (683)	27.7 (30.0)
Respiratory tract	1,041	5.3	614	20.7	604 (397)	19.1 (17.4)
Bloodstream	2,230	11.4	415	14.0	357 (258)	11.3 (11.3)
Other	3,390	17.3	497	16.8	836 (619)	26.4 (27.2)
Source of infection						
Community	10,658	55.7	1,169	40.5	1,105 (781)	35.6 (34.9)
Nosocomial	7,606	39.8	1,592	55.2	1,771 (1,284)	57.1 (57.4)
Nosocomial other admission[b]	870	4.5	123	4.3	224 (172)	7.2 (7.7)
Hospital size (number of beds)						
<200	5,568	28.1	688	23.0	697 (482)	21.8 (20.9)
200–500	8,030	406	1,256	41.9	1,297 (952)	40.5 (41.3)
>500	6,198	31.3	1,053	35.1	1,210 (873)	37.8 (37.8)
Ward						
Medical	8,950	45.5	1,352	45.3	914 (643)	28.8 (28.1)
Surgical	6,314	32.1	867	29.1	1,422 (1, 062)	44.7 (46.4)
Intensive care unit	1,462	7.4	415	13.9	526 (352)	16.5 (15.4)
Gynecology and obstetrics	635	3.2	47	1.6	48 (30)	1.5 (1.3)
Pediatric	1,623	8.3	206	6.9	210 (163)	6.6 (7.1)
Other	680	3.5	95	3.2	59 (38)	1.9 (1.7)

[a] Numbers in parentheses are specific for *Enterobacter cloacae.*

[b] Patients hospitalised who, at the time of the survey, presented a nosocomial infection acquired during a previous admission in the same or another hospital.

significantly associated to nosocomial infections, and the rates of fluoroquinolone resistance increased with the size of the hospital, or age of the patients, especially in patients older than one year. For the type of ward, a high rate of resistance was found in patients admitted to ICU (23.1%), and the lowest rates of resistance were found in paediatric (2.9%). For localisation of infection, UTI showed the highest rate of resistance (Table 2).

Enterobacter *species infections*

Nosocomial acquisition, ICU and pediatric wards, as well as bloodstream infections and UTI showed the highest prevalence of *Enterobacter* spp. resistance to

Table 2

Prevalence of resistance to third-generation cephalosporins and fluoroquinolones by microorganism and by characteristics of patient, infection, and hospital, Spain, 1999–2010

	Escherichia coli				*Klebsiella pneumoniae*				*Enterobacter* spp. *(Enterobacter cloacae)*[a]			
	Third-generation cephalosporin resistance		Fluoroquinolone resistance		Third-generation cephalosporin resistance		Fluoroquinolone resistance		Third-generation cephalosporin resistance		Fluoroquinolone resistance	
	Rate (in %)	p value	Rate (in %)	p value	Rate (in %)	p value	Rate (in %)	p value	Rate (in %)	p value	Rate (in %)	p value
Sex												
Male	10.2	0.016	27.7	<0.001	14.4	0.775	17.9	0.486	25.1 (26.8)	0.982 (.961)	10.3 (10.6)	0.116 (.314)
Female	9.2		24.7		14.0		16.9		25.1 (27.0)		8.5 (9.3)	
Age (in years)												
≤1	4.8	<0.001	5.8	<0.001	17.4	0.315	2.8	<0.001	29.8 (33.6)	0.274 (.206)	3.9 (5.1)	0.012 (.033)
1–15	7.1		6.7		5		10.0		18.8 (21.1)		4.2 (2.6)	
16–45	8.4		20.5		13.3		16.0		25.7 (28.9)		10.2 (10.6)	
46–65	9.8		25.6		13.8		17.7		23.1 (24.6)		8.4 (8.9)	
>65	10.5		30.1		14.4		19.3		25.5 (26.8)		10.8 (11.3)	
Localisation of infection												
Urinary tract	8.7	<0.001	26.7	<0.001	16.6	0.065	22.7	<0.001	28.3 (32.4)	0.002 (.005)	13.2 (16.2)	0.015 (.001)
Surgical site	10.5		22.9		15.1		14.1		25.9 (26.1)		7.6 (7.8)	
Respiratory tract	14.7		30.9		12.1		14.5		25.3 (28.7)		10.3 (10.1)	
Bloodstream	9.7		25.1		12.5		14.0		29.1 (30.6)		8.1 (8.5)	
Other	10.3		26.7		13.1		15.9		20.2 (22.1)		10.0 (10.5)	
Source of infection												
Community	7.9	<0.001	24.8	<0.001	8.6	<0.001	12.1	<0.001	20.8 (23.4)	<0.001 (.002)	9.4 (10.8)	0.947 (.686)
Nosocomial	11.6		27.3		17.9		21.0		28.0 (29.8)		9.8 (9.7)	
Nosocomial other admission[b]	14.7		32.2		20.3		18.7		23.2 (21.5)		9.8 (9.3)	
Hospital size (number of beds)												
<200	8.5	<0.001	25.6	0.012	13.7	0.016	15.6	0.038	24.0 (26.6)	0.238 (.157)	8.6 (8.9)	0.007 (.010)
200–500	8.9		25.4		12.5		16.4		24.1 (25.0)		8.2 (8.4)	
>500	11.9		27.4		16.6		19.7		26.7 (29.0)		11.7 (12.5)	
Ward												
Medical	10.4	<0.001	31.4	<0.001	13.5	0.036	19.1	<0.001	24.1 (26.1)	0.002 (.001)	12.4 (13.4)	<0.001 (<.001)
Surgical	9.5		24.2		13.1		14.5		22.6 (22.8)		8.6 (8.5)	
Intensive care unit	13.3		27.0		18.3		23.1		31.6 (36.4)		10.3 (11.6)	
Gynecology and obstetrics	4.4		10.6		4.3		6.4		22.9 (23.3)		0.0 (0)	
Pediatric	5.5		5.4		16.0		2.9		28.6 (33.1)		2.9 (3.1)	
Other	7.2		32.6		16.8		30.5		30.5 (36.8)		16.9 (18.4)	

[a] Numbers in parentheses are for *Enterobacter cloacae*.

[b] Patients hospitalised who, at the time of the survey, presented a nosocomial infection acquired during a previous admission in the same or other hospital.

third-generation cephalosporins. For fluoroquinolones resistance, the factors found associated were age, hospital size, ward type and localisation of infection (Table 2).

Average of resistance for *E. cloacae* and *E. aerogenes* did not differ significantly (26.9% vs 24.1%, RR:1.12, 95% CI: 0.95–1.31, and 10.1% vs 9.4%, RR:1.08 95% CI:0.81–1.43, for resistance to third-generation cephalosporins and to fluoroquinolones respectively).

Regarding the main species of *Enterobacter* (*E. cloacae*) significant differences were found in rates of resistance to third-generation cephalosporins, for localisation of infection (highest in UTI, 32.4%), nosocomial (29.8%) and type of ward (highest in ICU, 36.4%), and in rates of resistance to fluoroquinolones for increasing age, size of the hospital, localisation of infection (highest in UTI, 16.2%), and type of ward (highest in medical ward patients, 13.4%) (Table 2).

Trends in antimicrobial use and resistance

Antimicrobial use (number of patients receiving antibiotic/100 admitted patients) increased steadily for fluoroquinolones from 5.8% in 1999 to 10.2% in 2010 ($p<0.001$), but no significant trend was observed for third-generation cephalosporins use (from 6.4% in 1999 to 5.9% in 2010) (Figure 1).

Proportion of resistance to third-generation cephalosporins increased significantly for *E. coli* infections (from 4.6% to 15.4%, $p<0.01$) and for *K. pneumoniae* infections (from 3.9% to 20.8%, $p<0.01$) with a more pronounced increase since year 2004. Nevertheless third-generation cephalosporins resistance for *Enterobacter* spp. did not show a trend, and ranged from 22.5% to 31% with a mean rate of 24.3% (Figure 2).

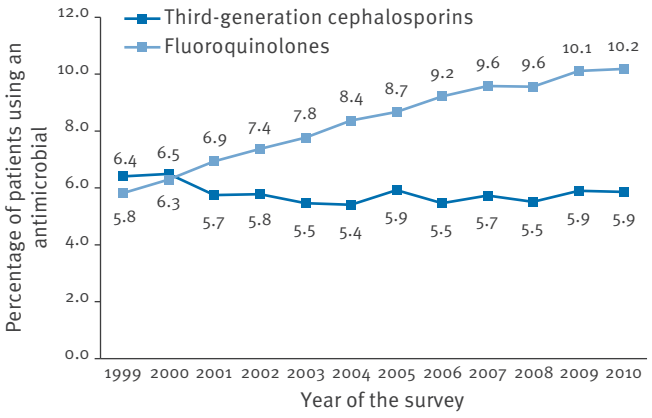
Proportion of resistance to fluoroquinolones increased significantly for *E. coli* infections (from 15.9% to 29.6%, $p<0.01$), and to a minor extent for *Enterobacter* spp. (from 6.4% to 14.9%, $p<0.01$). For *K. pneumoniae* there was a great increase (from 5.5% to 22.4%, $p<0.01$), also more pronounced since 2004 (Figure 3).

Proportion of co-resistance to fluoroquinolones and third-generation cephalosporins increased significantly for *E. coli* infections (from 1.6% to 11.3%, $p<0.01$), for *Enterobacter* spp. (from 4.8% to 9.5%, $p<0.01$), and to a higher extent for *K. pneumoniae* infections (from 0.8% to 14.4%, $p<0.01$) and more pronounced during the period from 2004 to 2007 (Figure 4).

We found strong correlations for rates of *E. coli* resistant to fluoroquinolones, resistant to third-generation cephalosporins, or co-resistant to third-generation cephalosporins and fluoroquinolones, by the rate of use of fluoroquinolones ($R=0.97$, $p<0.01$; $R=0.94$, $p<0.01$; and $R=0.96$, $p<0.01$, respectively) (Figure 5A).

Figure 1

Annual use of fluoroquinolones and third-generation cephalosporins, Spain, 1999–2010



Use of fluoroquinolones or third-generation cephalosporins is defined as the number of patients receiving either type of antimicrobial per 100 patients on the day of the annual survey.

Figure 2

Annual rates of *Enterobacteriaceae* resistant to third-generation cephalosporins, Spain, 1999–2010

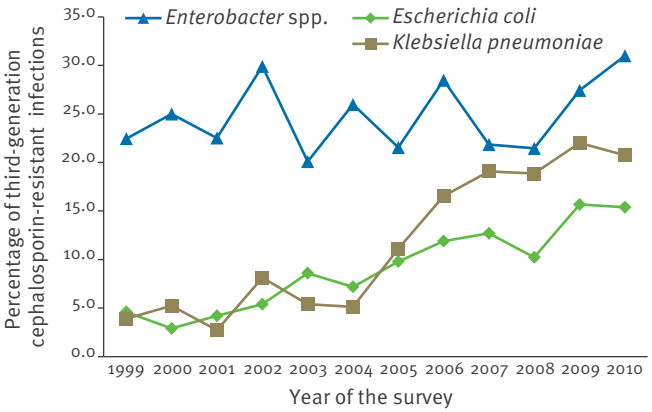
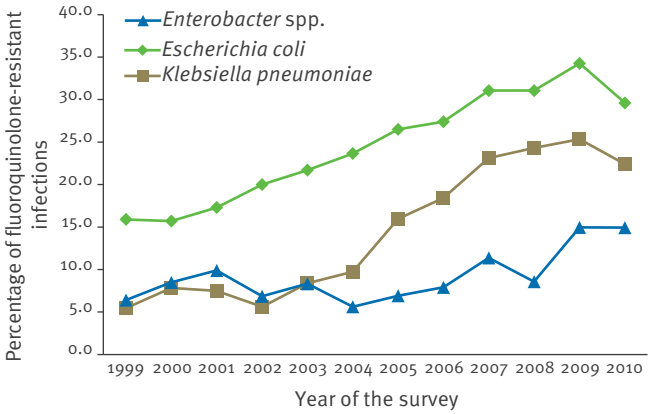


Figure 3

Annual rates of *Enterobacteriaceae* resistant to fluoroquinolones, Spain, 1999–2010



Also we found strong correlations for rates of *K. pneumoniae* resistant to fluoroquinolones, to third-generation cephalosporins, or co-resistant to third-generation cephalosporins and fluoroquinolones, by the rate of use of fluoroquinolones (R=0.92, p<0.01; R=0.91, p<0.01; and R=0.92, p<0.01, respectively) (Figure 5B), but these correlations could not be found by the use of third-generation cephalosporins. No significant correlations could be found for *Enterobacter* spp. resistant to third-generation cephalosporins and use of third-generation cephalosporins or of fluoroquinolones. This was also the case for *Enterobacter* spp. resistant to fluoroquinolones and use of third-generation cephalosporins or fluoroquinolones.

Discussion

In the present study, we observed an increase in rates of resistance to third-generation cephalosporins and fluoroquinolones in the past twelve years in the main *Enterobacteriaceae* causing infections in hospitalised patients in Spain. This high rate of antibiotic resistance could increase the risk of inappropriate empirical therapy in hospitalised patients with potentially serious infections. In Spain, fluoroquinolones and third-generation cephalosporins can be used empirically for both nosocomial and community-acquired pneumonias and UTIs. The increase in resistance to fluoroquinolones and third-generation cephalosporins nevertheless differed among *Enterobacteriaceae* representatives.

Enterobacter spp. (mainly *E. cloacae*) displayed the highest rates of resistance to third-generation cephalosporins and the rates did not increase over time, but by 2010 *E. coli* and *K. pneumoniae* isolates were 3.3 and 5.3 times more frequently resistant respectively than in 1999. The difference in resistance for *Enterobacter* spp. could be due to the fact that the main mechanism of resistance to third-generation cephalosporins for *Enterobacter* spp. is overproduction of AmpC beta-lactamases, whereas for *E. coli* and *K. pneumoniae* the main resistance mechanism is extended-spectrum beta-lactamases (ESBL) that has been related to antibiotic usage [9].

Overall, an increase in resistance to fluoroquinolones was observed for all three microorganisms reaching levels of resistance of 30%, 22%, and 15%, for *E. coli*, K pneumoniae, and *Enterobacter* spp. respectively. This tremendous increase in resistance was encompassed over the same time period by a 75% increase in the use fluoroquinolones in the hospitalised population. Furthermore, strong correlations were found for the use of fluoroquinolones inside the hospital and resistance to fluoroquinolones, to third-generation cephalosporins, or co-resistance to both groups of the latter antimicrobials for *E. coli* and *K. pneumoniae*. From a microbiological point of view, the increase of

Figure 4

Annual rates of *Enterobacteriaceae* co-resistant to fluoroquinolones and third-generation cephalosporins, Spain, 1999–2010

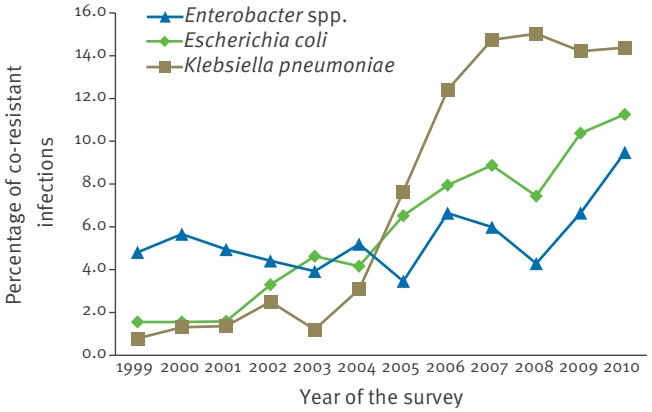
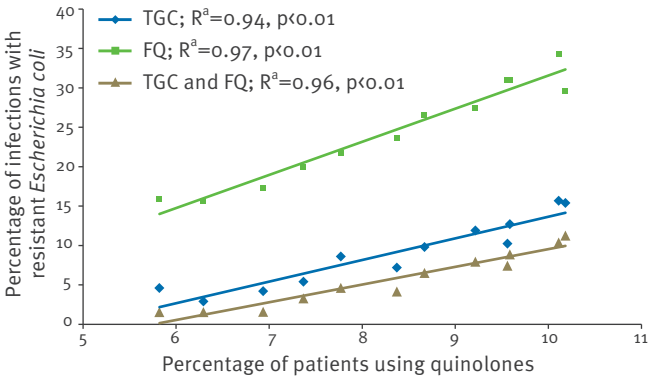


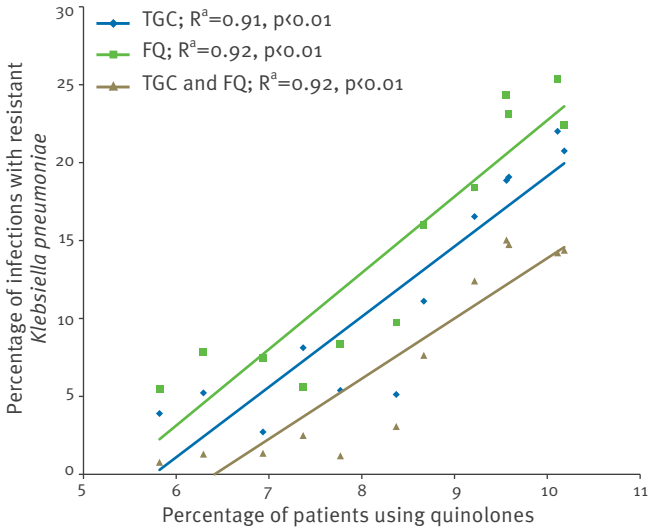
Figure 5

Correlation between annual rate of fluoroquinolone use and resistance to fluoroquinolones, third-generation cephalosporins, or fluoroquinolones and third-generation cephalosporins, Spain, 1999–2010

A. *Escherichia coli*



B. *Klebsiella pneumoniae*



FQ: Fluoroquinolones; TGC: Third-generation cephalosporins.

[a] Pearson correlation coefficient.

resistance to fluoroquinolones has been associated with mutations in the topoisomerases [10] and more recently by the acquisition of plasmid mediated fluoroquinolones resistance genes such as *qnr*, *aac(6`)-I-cr* or *qep* particularly in ESBL producing isolates [11]. Although the use of third-generation cephalosporins did not increase over the time period surveyed, use of this antimicrobial was maintained. The continuing use of third-generation cephalosporins could have contributed to an increase in the rate of resistance of *E. coli* and *K. pneumoniae* to third-generation cephalosporins. In the hospital environment, the increase of fluoroquinolone use may also have allowed cephalosporin-resistant *Enterobacteriaceae* to acquire additional resistance to fluoroquinolones. A co-selection process of co-resistance might have occurred [12].

In recent years, other studies have observed Spain as one of the countries in Europe with the highest use of fluoroquinolones outside the hospital [13]. This may have a role in co-selection and co-resistance, and potential clonal expansion influencing the results of this study. The observed differences in third-generation cephalosporin resistance among *Enterobacteriaceae* representatives could be related to the main resistance mechanism in the different studied bacteria (ESBL in *E. coli* and K. pneumonaie or AmpC hyperproduction in *Enterobacter* spp.) [14].

Not surprisingly, rates of resistance to third-generation cephalosporins were higher for ICU infected patients than on average, but the highest rates of fluoroquinolone resistance were found outside the ICUs (Table 2) probably related to the widespread use of this class of antimicrobial outside the ICUs.

It is important to note that resistance to antimicrobials can differ depending of the site of the infection. For *E. coli* the main resistance to third-generation cephalosporins was found in respiratory tract infections (14.7%), infections that are rarely caused by *E. coli* outside the critical care units [15], a much higher rate than that for bloodstream infections (9.7%, p<0.001). Nevertheless, for *Enterobacter* spp. the highest rates of third-generation cephalosporin resistance were found for bloodstream infections (29.1%). Regarding resistance to fluoroquinolones the highest resistance in *E. coli* was for respiratory tract infections, while for *K. pneumoniae* and *Enterobacter* spp. was for UTI. It is important to keep in mind these differences when comparing to rates derived from a single site of infection.

It is also of note that for all these enterobacterial infections more than 50% (56% for *E. coli* and 41% and 36% for *K. pneumoniae* and *Enterobacter* spp., respectively) are community-acquired infections, that 88% of them are not bloodstream infections, and that looking for resistance only in nosocomial or bloodstream infections, in studies of antimicrobial resistance, can omit an important and valuable information for the development of prevention strategies.

Regarding co-resistance to both fluoroquinolones and third-generation cephalosporins, *Enterobacter* spp., *E. coli* and *K. pneumoniae* increased significantly their rates of co-resistance with time, but the most pronounced increase could be observed for *K. pneumoniae* reaching rates of co-resistance of almost 15% in the 2007–2010 period. It has been reported that many enterobacteria strains resistant to third-generation cephalosporins are also resistant to fluoroquinolone, and that this co-resistance could be mediated by plasmids harbouring both quinolone resistance and ESBL genes or presence of different plasmids harbouring these resistance genes [16]. It is remarkable that the greatest increase in co-resistance for these microorganisms, and to some extent for the resistance to fluoroquinolones and to third-generation cephalosporins, started around year 2004. These facts do not seem to be related to the antimicrobial use of fluoroquinolones or third-generation cephalosporins in hospitals, neither to any other fact known by us, and remains to be elucidated. It should be interesting to study the relationship of these facts with the consumption of other antimicrobial, such as amoxicillin-clavulanic, broadly used in Spain, since new formulations, for oral and parenteral routes, with increased dosage started to be marketed in Spain at the end of 2003.

To which extent rates of resistance derived from point prevalence studies can be overestimated when these rates of resistance are derived from an augmented length of infectious state attributable to antimicrobial resistance, needs further evaluation. When comparing data from our study to those reported by the European Antimicrobial Resistance Surveillance Network (EARS-net, formerly EARSS) for the period 2007–2009 in Spain, only slight differences were found (values for EARS-net and EPINE: non-suceptible *E. coli* to fluoroquinolones 32.3% vs 28.7%, or to third-generation cephalosporins 9.5% vs 10.7%, non-susceptible *K. pneumoniae*: to fluoroquinolones 17.2% vs 20.8%, to third-generation cephalosporins 11.1% vs 16.0%) [17]. Nevertheless, it should be taken into account that EARS-net records data to a net of national surveillance systems only on invasive infections, from voluntary clinical laboratories in Spain (between 29 to 33 and between 14 to 33 laboratories reporting for *E. coli* and *K. pneumoniae*, respectively), as opposed to the data from around 265 hospitals per year in EPINE, and that huge variations are found among laboratories reflecting wide local variations in rates of resistance [18].

There are some limitations for our study. As hospitals participated on a voluntary or compulsory basis, but were not selected at random, representativeness applies to regions where the study is mandatory but can not be warranted at the national level. On the other hand, this prevalence series represents more than half of the population hospitalised in acute care centres in Spain on a given day, and most data come from hospitals that have regularly participated in the survey every year so concerns about representativeness are

diminished. Another limitation of our work is related to the non-experimental nature of the study so that to which extent this increase in antimicrobial resistance can be attributable to the increase in the use of fluoroquinolones cannot be concluded from the results of this ecological study but those results points out to this direction. One of the concerns about generalising our data is seasonality. As the survey was performed every year during May, seasonal variations in time could not be assessed. However, the fact that we performed the survey in the same season each year, although precluding a study of seasonality, allowed us to measure trends. Finally, point prevalence studies prove to be useful to monitor trends on rates of resistance and antimicrobial use at a national level, and can provide valuable information for comparisons among European Union Member States. Furthermore the information on antimicrobial use and resistance gathered by national prevalence surveys, linked to patient and infection characteristics, not focused exclusively on invasive infections, can complement that of much rigorous databases, from the microbiological point of view, such as EARS-net and could help to develop national strategies to prevention.

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