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New Delhi Metallo-beta-lactamase around the world: An eReview using Google Maps

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Gram-negative carbapenem-resistant bacteria, in particular those producing New Delhi Metallo-beta-lactamase-1 (NDM-1), are a major global health problem. To inform the scientific and medical community in real time about worldwide dissemination of isolates of NDM-1-producing bacteria, we used the PubMed database to review all available publications from the first description in 2009 up to 31 December 2012, and created a regularly updated worldwide dissemination map using a web-based mapping application. We retrieved 33 reviews, and 136 case reports describing 950 isolates of NDM-1-producing bacteria. K*lebsiella pneumoniae* (n= 359) and *Escherichia coli* (n=268) were the most commonly reported bacteria producing NDM-1 enzyme. Several case reports of infections due to imported NDM-1 producing bacteria have been reported in a number of countries, including the United Kingdom, Italy, and Oman. In most cases (132/153, 86.3%), patients had connections with the Indian subcontinent or Balkan countries. Those infected were originally from these areas, had either spent time and/or been hospitalised there, or were potentially linked to other patients who had been hospitalised in these regions. By using Google Maps, we were able to trace spread of NDM-1-producing bacteria. We strongly encourage epidemiologists to use these types of interactive tools for surveillance purposes and use the information to prevent the spread and outbreaks of such bacteria.

Introduction

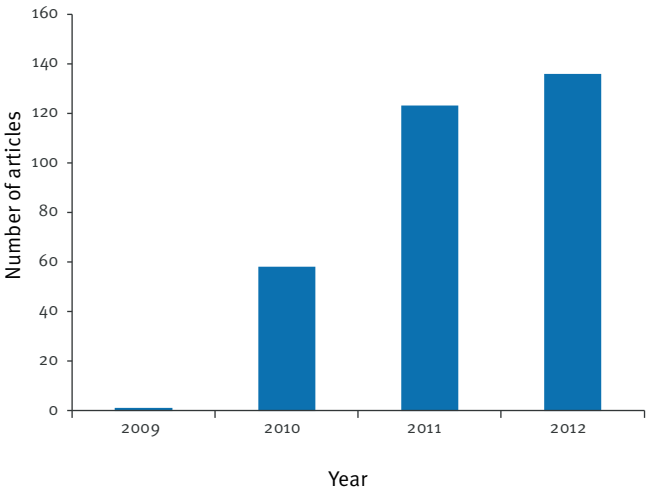
New Delhi Metallo-beta-lactamase-1 (NDM-1) is the most recently discovered transferable molecular class B beta-lactamase. Unlike class A, C and D beta-lactamases, NDM-1 has zinc ions at its active site, and it can hydrolyse all beta-lactam antimicrobials except for monobactam [1-3]. Moreover, most NDM-1-positive bacteria are resistant to a wide variety of other antimicrobial classes and carry several additional resistance mechanisms for example to aminoglycosides, fluoroquinolones, macrolides and sulfonamides, leaving few or no therapeutic options [4-8]. The putative original source of the bla_{NDM-1} gene could be from a chromosome of plant pathogens, such as *Pseudoxanthomonas* and related bacteria that are widespread in the environment [9].

The first published reports of infections involved individuals who had received medical care in India. The precise geographic origin and the time of the first appearance of the bla_{NDM-1} genes are unknown, however. The first NDM-1-producing bacteria were isolated from a Swedish resident of Indian origin who contracted a urinary tract infection caused by carbapenem-resistant *Klebsiella pneumoniae* while he was in New Delhi in late 2007, hence the name [10]. At present, most bacteria isolated worldwide have originated from people colonised/infected (with or without showing infection symptoms) on the Indian subcontinent who have then traveled elsewhere [3,11]. However, it is presumed that there are other reservoirs of colonised/infected patients in the Balkan countries [12]. There is also an unknown burden in the Middle East, where people often travel to and from the Indian subcontinent [13].

NDM-1-producing bacteria have been recovered from many infection sites; they have been found in patients with urinary tract infections, pneumonia, septicaemia, wound infections and device-associated infections [7,14,15]. Both hospital- and community-acquired infections have been reported [7,14,16]. The following factors have influenced the geographically widespread emergence of these NDM-1-producing bacteria: the increase in long-distance travel [17], the increase in international travel to access medical care [18] and widespread access to broad spectrum antibiotics. The latter is due to the fact that in many countries, antibiotics can be obtained without a prescription because of the strong economic incentives to sell and use them [19].

Figure 1

Number of articles retrieved from PubMED database using keywords 'NDM-1' or 'New Delhi Metallo-beta-lactamase-1' per year, 1 December 2009–31 December 2012 (n=235)



Given the volume of international travel, the quality of hygienic standards in many countries, and the number of humans carrying NDM-1-producing bacteria, it is likely that these bacteria will continue to spread worldwide [15]. There has been an increase in the number of articles about the 'New Delhi Metallo-beta-lactamase-1' enzyme added to the PubMed database since 2010 (Figure 1), but the current spread of NDM-1-prodcucing bacteria is likely broader than the published reports suggest.

To conduct an eReview of all published isolates worldwide as of end 2012, we used in this article the Google Maps application to simplify and accelerate access to documentation, organise information about published isolates of NDM-1-producing bacteria and provide real-time information to the scientific and medical community about published isolates of NDM-1-producing bacteria around the world. Few studies have used this type of automated system to investigate, in real time, web-based electronic reports for the purpose of monitoring the spread of infectious diseases caused by influenza A (H1N1) and Dengue viruses [20,21]. Google Maps is a widely available, free of charge, and extremely powerful tool for visualisation with a simple, intuitive interface that requires little training or experience to use it. It can be run on any conventional desktop computer or laptop, and there is also a Google Maps application available for mobile phones [22].

Because a visual representation of scientific data is more informative than a written description, this article describes the development of an internet-based mapping and geo-referencing application for tracking the worldwide dissemination of NDM-1-producing bacteria as an example of this application. We analysed in this article the medical literature from the first case report in December 2009 until 31 December 2012.

Methods

Literature search in the PubMed database

We started by retrieving all published articles from the PubMed database using 'NDM-1' and 'New Delhi Metallo-beta-lactamase-1' as keywords, from the first case report in 2009 until 31 December 2012. We included in our analysis only the first publications that reported on isolates of NDM-1-producing bacteria. We excluded all consecutive publications about the same isolates with descriptions of genomic or protein analysis or others types of analysis. After reading and analysing the full article, we specifically extracted the year of detection of the isolates, their geographic location (city and country), the NDM-1-producing bacterial species, the number of published isolates, the type of case reports, the title and the full reference for the published article, the link to the isolates description in PubMed database.

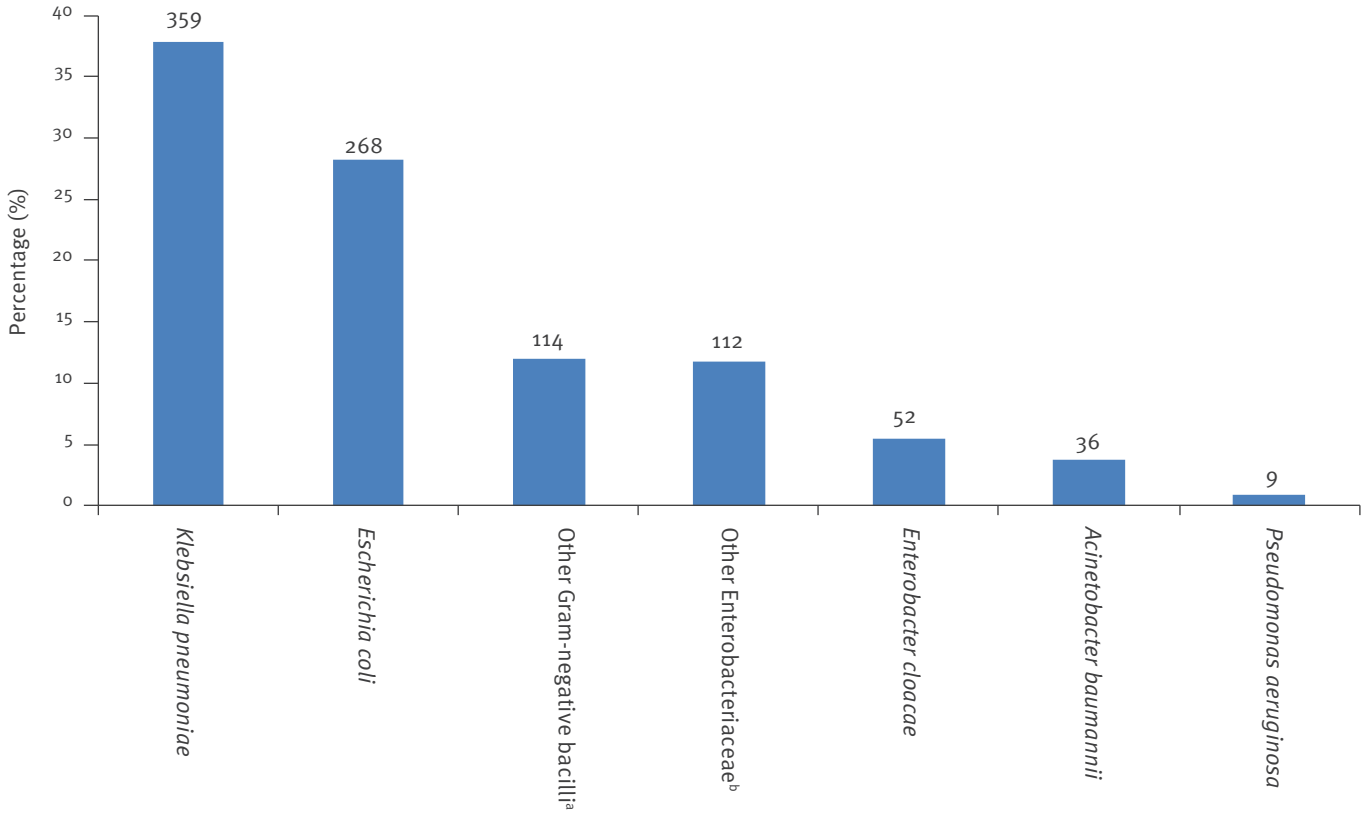
A case report of NDM-1-producing bacteria was defined as a patient from whom one or more Gram-negative bacteria had been isolated that produce NDM-1 or when an isolate from the environment contains NDM-1-producing bacteria, with the test result confirmed by an expert laboratory. We categorised case reports into five different types: (i) imported human infection case reports (NDM-1-producing bacteria isolated from patients with a history of recent travel or contact with healthcare facilities abroad before the detection of $bla_{\text{NDM-1}}$ gene), (ii) autochthonous human infection case reports (reports of patients with an infection caused by NDM-1-producing bacteria who did not have contact with a travel-associated case), (iii) autochthonous human carriage case reports(carriage of NDM-1-producing bacteria in patients who did not have contact with a travel-associated case), (iv) autochthonous environmental case reports (a description of NDM-1-producing bacteria in the hospital or the external environment) and (v) autochthonous human carriage and environmental case reports (a description of the two types of cases in the same article).

Construction of Google Maps

We used Google Maps to create an electronic map depicting the geographic locations of case reports of NDM-1-producing bacteria listed in our database. Google provides full documentation for Google Maps, tutorials and other materials to help users take full advantage of the application (https://maps.google.com). The locations on the map were tagged using different symbols for each type of report of NDM-1-producing bacteria. Clicking the tags will provide a display of the important information about the selected article (the same information stored in the columns in the database). If there are several tags within close proximity to one another, the tags expand outward to facilitate selection of a single tag. Google Maps navigation tabs in the upper left of the screen can be used to zoom in on an area of interest. Alternatively, one can double-click on one of the locations in the table of

Figure 2

Distribution of New Delhi Metallo beta-lactamase-producing bacteria species, 1 December 2009–31 December 2012 (n=950)



[a] *Acinetobacter pittii* (n=27), *Acinetobacter lwoffii* (n=20), *Acinetobacter* sp. (n=20), *Pseudomonas aeruginosa* (n=9), *Moraxella* ssp.(n=8), *Comamonas testosteroni* (n=7), *Pseudomonas* sp. (n=7), *Stenotrophomonas maltophilia* (n=5), *Vibrio cholerae* (n=3), *Achromobacter* spp. (n=2), *Acinetobacter johnsonii* (n=2), *Alcaligenes faecalis* (n=2), *Pseudomonas pseudoalcaligenes* (n=2), *Pseudomonas putida* (n=2), *Acinetobacter junii* (n=1), *Acinetobacter ursingii* (n=1), *Aeromonas caviae* (n=1), *Kingella denitrificans* (n=1), *Methylobacterium* spp. (n=1), *Pseudomonas oryzihabitans* (n=1), *Suttonella indologenes* (n=1)

[b] *Citrobacter* spp. (n=44), non-determined Enterobacteriaceae (n=15), *Klebsiella* spp. (n=10), *Morganella morganii* (n=8), *Enterobacter* spp. (n=7), *Providencia rettgeri* (n=6), *Klebsiella oxytoca* (n=5), *Proteus mirabilis* (n=4), *Providencia stuartii* (n=3), *Enterobacter aerogenes* (n=2), *Proteus* spp. (n=2), *Citrobacter braakii* (n=1), *Proteus vulgaris* (n=1), *Providencia* spp. (n=1), *Salmonella enterica* (n=1), *Salmonella* spp. (n=1), *Shigella boydii* (n=1)

contents on the left-hand side of the screen to access information about the selected article.

Data retrieved were stored and analysed in Excel (Microsoft, Redmond, WA, USA).

Results

The eReview display

To visualise the case reports of NDM-1-producing bacteria detected that have appeared around the world since the first description, we developed a Google Maps application as described in the methods section that is regularly updated and freely available on line at the following website: http://www.mediterranee-infection.com/article.php?laref=318&titre=new-delhi-metallo-lactamase-around-the-world. As soon as an article with the keyword 'NDM-1' or 'New Delhi Metallo-beta-lactamase-1' is added to the PubMed database, we automatically receive an alert by email. In less than 10 minutes, we are able to analyse the article, extract the relevant information about the published isolates, add it to our own database and update the map so that the information is freely accessible. Other NDM enzymes are not included in the manuscript but have been added in the Google map website.

Distribution of case reports of New Delhi Metallo-beta-lactamase-1-producing bacteria

From its first description in 2009 through 31 December 2012, there have been 33 reviews describing the *bla*NDM-1 gene [3,11,14,15,19,23-50], and 136 case reports in the PubMed database, reporting on 950 isolates of NDM-1-producing bacteria from around the world. There have been 66 articles describing imported human infection isolates with 153 (16.1%) isolates of NDM-1-producing bacteria; 57 articles describing autochthonous human infection isolates with 571 (60.1%) isolates of NDM-1-producing bacteria; and 13 articles describing autochthonous human carriage

Table 1

Bacteria producing New Delhi Metallo-beta-lactamase-1 enzyme reported worldwide by frequency, 1 December 2009 - 31 December 2012 (n=950)

Species	Number of isolates	Percentage of total
Klebsiella pneumoniae	359	37.8
Escherichia coli	268	28.2
Enterobacter cloacae	52	5.5
Citrobacter spp.	44	4.7
Acinetobacter baumannii	36	3.8
Acinetobacter pittii	27	2.8
Acinetobacter lwoffii	20	2.1
Acinetobacter sp.	20	2.1
Non-determined Enterobacteriaceae	15	1.6
Klebsiella spp.	10	1.0
Pseudomonas aeruginosa	9	0.9
Moraxella spp.	8	0.8
Morganella morganii	8	0.8
Comamonas testosteroni	7	0.7
Enterobacter spp.	7	0.7
Pseudomonas sp.	7	0.7
Providencia rettgeri	6	0.6
Klebsiella oxytoca	5	0.5
Stenotrophomonas maltophilia	5	0.5
Proteus mirabilis	4	0.4
Providencia stuartii	3	0.3
Vibrio cholerae	3	0.3
Achromobacter spp.	2	0.2
Acinetobacter johnsonii	2	0.2
Alcaligenes faecalis	2	0.2
Enterobacter aerogenes	2	0.2
Proteus spp.	2	0.2
Pseudomonas pseudoalcaligenes	2	0.2
Pseudomonas putida	2	0.2
Acinetobacter junii	1	0.1
Acinetobacter ursingii	1	0.1
Aeromonas caviae	1	0.1
Citrobacter braakii	1	0.1
Kingella denitrificans	1	0.1
Methylobacterium spp.	1	0.1
Proteus vulgaris	1	0.1
Providencia spp.	1	0.1
Pseudomonas oryzihabitans	1	0.1
Salmonella enterica	1	0.1
Salmonella spp.	1	0.1
Shigella boydii	1	0.1
Suttonella indologenes	1	0.1
Total	**950**	**100.0**

and environmental case reports, reporting 172 (18.1%) and 54 (5.7%) isolates of NDM-1-producing bacteria, respectively.

Klebsiella pneumoniae (n= 359) and *Escherichia coli* (n=268) were the most commonly described NDM-1-producing bacteria (Figure 2). The bla_{NDM-1} gene has also been recorded in Enterobacteriaceae other than *K. pneumoniae* and *E. coli* (Table 1); NDM-1-production has been found in clinical *Acinetobacter baumannii* (n=36), *Pseudomonas aeruginosa* (n=9) isolates and in a wide variety of non-fermenting Gram-negative species (Table 1).

Distribution of autochthonous case reports of New Delhi Metallo-beta-lactamase -producing bacteria by country

In India, NDM-1-producing bacteria were retrieved from patients in many different cities, including Chennai, Guwahati, Varanasi, Mumbai, Haryana, Kolkata, New Delhi, Pune, Bangalore, and Assam. There have been 374 isolates of NDM-1-producing bacteria responsible for autochthonous human infection [6,7,51-66]; 21 isolates of NDM-1-producing bacteria were responsible for autochthonous human carriage [54,67,68], and 22 isolates of NDM-1-producing bacteria were identified in the environment [54,69]. In Pakistan, 32 isolates of NDM-1-producing bacteria were responsible for autochthonous human infection described in nine cities [66, 70], and 101 isolates of NDM-1-producing bacteria were responsible for autochthonous human carriage [70,71]. In China, 16 isolates of NDM-1-producing bacteria were responsible for autochthonous human infection described in eight cities [72-81], 49 isolates of NDM-1-producing bacteria were responsible for autochthonous human carriage [72,82,83], and 30 isolates of NDM-1-producing bacteria were identified in the environment [84-86].For the remainder countries, 149 isolates of NDM-1-producing bacteria responsible for autochthonous human infection have been identified in the United Kingdom (n=23) [59,66], Canada (n=18) [87-90], Bangladesh (n=17) [91,92], Singapore (n=15) [93-95], Israel (n=10) [96,97], Serbia (n=8) [98,99], Kenya (n=7) [100], Kosovo (n=7) [101], Thailand (n=6) [102], France (n=4) [103-105], Japan (n=4) [106-108], Morocco (n=4) [109,110], South Korea (n=4) [111], Sweden (n=4) [59], Switzerland (n=3) [112], Afghanistan (n=2) [113], Guatemala (n=2) [114], South Africa (n=2) [115], Vietnam (n=2) [116], United Arab Emirates (n=2) [117], Iran (n=1) [118], Mauritius (n=1) [119], Netherlands (n=1) [120], Spain (n=1) [121], and Taiwan (n=1) [122]. Details are included in Figure 3A. Table 2 summarises the distribution of NDM-1-producing bacteria, grouped according to the type of autochthonous case reports in 29 countries. The year of the first description is indicated for each country. The first NDM-1 producing bacteria causing a human infection was isolated in India in 2006 [6], followed by Kenya in 2007 [100] and the Netherlands in 2008, the latter a putative secondary transmission [120].

Table 2

Distribution of New Delhi Metallo-beta-lactamase-1-producing bacteria reported in autochthonous case reports by country, 1 December 2009–31 December 2012 (n=797)

Type of case reports	Country	Cities	Number of isolates	First description	References
Human infection	Afghanistan	Kabul	2	2011	[113]
	Bangladesh	Dhaka	17	2008	[91, 92]
	Canada	Brampton, Toronto, Winnipeg	18	2009-2010	[87-90]
	China	Beijing, Changsha, Chongqing, Fujian, Guangzhou, Hangzhou, Hebei, Hong Kong	16	2009-2012	[72-81]
	France	Bordeaux, Lyon, Toulon	4	2011	[103-105]
	Guatemala	Not available	2	2011	[114]
	India	Assam, Bangalore, Chennai, Guwahati, Haryana, Kolkata, Mumbai, New Delhi, Pune, Varanasi	374	2006-2007	[6, 7, 51, 51-66]
	Iran	Tehran	1	2011	[118]
	Israel	Jerusalem, Tel Aviv	10	2010	[96, 97]
	Japan	Saitama, Tokyo	4	2010	[106-108]
	Kenya	Nairobi	7	2007-2009	[100]
	Kosovo*	Pristina	7	2010	[101]
	Mauritius	Quatre Bornes	1	2009	[119]
	Morocco	Rabat, Taza	4	2011	[109, 110]
	The Netherlands	Enschede	1	2008	[120]
	Pakistan	Charsadda, Faisalabad, Gujrat, Hafizabad, Karachi, Khan, Lahore, Rahim Yar, Sheikhupura	32	2009	[66, 70]
	Serbia	Belgrade	8	2010	[98, 99]
	Singapore	Singapore	15	2011	[93-95]
	South Africa	Johannesburg	2	2011	[115]
	South Korea	Seoul	4	2010	[111]
	Spain	Madrid	1	2012	[121]
	Sweden	Stockholm	4	2011	[59]
	Switzerland	Geneva	3	2009-2010	[112]
	Taiwan	Taipei	1	2011	[122]
	Thailand	Khon Kaen	6	2010	[102]
	Vietnam	Hanoi	2	2010	[116]
	United Arab Emirates	Abu Dhabi	2	2011	[117]
	United Kingdom	10 cities (not available)	23	2011	[59, 66]
Human carriage	Cameroon	Douala	1	2012	[195]
	China	Beijing, Changsha	49	2011	[72,82, 83]
	India	Chennai, Guwahati, Kolkata	21	2009	[54, 67, 68]
	Pakistan	Rawalpindi	101	2010	[70, 71]
Hospital or the external environment	China	Beijing, Chengdu	30	2012	[84-86]
	India	Kolkata, New Delhi	22	2010	[54, 69]
	Vietnam	Hanoi	2	2011	[116]

* This designation is without prejudice to positions on status, and is in line with UNSCR 1244/99 and the ICJ Opinion on the Kosovo declaration of independence.

Distribution of imported case reports of New Delhi Metallo beta-lactamase-producing bacteria by country

Several imported isolates of NDM-1-producing bacteria have been reported in a number of countries in different geographical locations, but most of them have been reported in the United Kingdom (n=44) [7,123-125] (Table 3). The first imported NDM-1-producing bacteria was isolated in 2007 in Germany [126], followed by two isolates in 2008 in the United Kingdom [7] and the Netherlands, respectively [127]. In most of the cases, patients had connections to other countries or regions such as the Indian subcontinent (n=121) [7,57, 104,106,109,123-125,128-162], the Balkan (n=11) [8,112,131,163-168] , Africa (n=10)[117,123,160,165,169-174] , the Middle East (n=6) [175-178], and East Asia (n=5)[179,180] . The patients originated from these areas, had spent time or been hospitalised there, or they might have been secondarily linked to other hospitalised patients who had recently returned from these areas. Figure 3B shows the putative countries of origin for the imported isolates of NDM-1-producing bacteria. The majority of these patients (61.2%) had been previously admitted to hospitals in another country because of an accident or an illness that occurred during their travel, although a minority of patients was traveling for medical reasons.

Discussion

The data presented indicate a worldwide increase in the spread of NDM-1-producing bacteria and other carbapenemase-producing bacteria [2,7,181]. In this study, we describe 950 isolates of NDM-1-producing bacteria from different types of case reports in 55 countries between 2006 and 31 December 2012, with the majority of isolates of NDM-1-producing bacteria from India, Pakistan and China. It is probable that the number of published NDM-1-producing bacteria underestimates the true number of cases infected/colonised with NDM-1-producing bacteria because most countries do not perform systematic surveillance for such infections with highly resistant bacteria and many bacteria are not tested for the production of NDM-1 enzyme. In some cases, the patient is asymptomatic, so only colonised. In addition, microbiological guidance on the detection and the identification of carbapenemase-producing bacteria is only available in a minority of countries, including the European Union [15,41]. The highest concentration of NDM-1-producing bacteria per million square kilometers of land was found between 30° and 60° northern latitude, with the main hotspots on the Indian subcontinent and in the Balkan countries. Moreover, the majority of the imported isolates described in our survey using published information to display the geographical occurrence of NDM-1, involved patients with a history of recent travel or hospital admission on the Indian subcontinent or in Balkan countries [4,7,15]. In 2008, India and Pakistan received an estimated five million visitors, and an estimated 10 million residents migrated from these countries which amount to a movement and dispersion of 15 million people to third countries [14]. It should be also noted that for some cases travel alone was sufficient to acquire NDM-1-producing bacteria [182].

In view of this situation, we believe that an immediate response to the emergence of NDM-1-producing bacteria and other carbapenemases should be an urgent priority worldwide. At a local level, patients with a history of travel to or originating from high-risk countries or areas should be screened for NDM-1-producing bacteria [126,127,183,184]. This screening should prevent the development of onward transmission and potential outbreaks and help to optimise the antibiotic therapy. At the international level, the response to growing multidrug resistance of Gram-negative bacteria should be the implementation of a worldwide surveillance network to discover and report emerging resistance traits [29]. To the best of our knowledge, this study is the first that used Google Maps as an interactive and free tool to document all isolates of NDM-1-producing bacteria worldwide. This tool could be also used to document occurrence and spread of other antibiotic resistance genes. It offers a new way to monitor genes responsible for antibiotic resistance, unlike other works that report on the bacteria responsible for infectious disease. Such a development is important because we are now witnessing outbreaks of resistance genes, not bacteria.

Google Maps can be advantageous to the scientific and medical community for a number of reasons. It facilitates (i) counting the isolates producing antibiotic resistance enzymes, (ii) estimating the prevalence of each bacterial species, (iii) differentiating between different types of case reports, (iv) visualising the relationship between the circulation of antibiotic resistance genes and the worldwide human traffic patterns, (v) identifying the origin and reservoir of the antibiotic resistance gene, and finally (vi) communicating information about the local and worldwide dissemination of antibiotic resistance genes in real time. The advantages of Google Maps also include the immediate access to the PubMed publications from the link in the case report description and the real-time update of the map as soon as an article is added on the PubMed database. Google Maps represents a new generation of interactive review capability; it is easy to use, and it is accessible everywhere by everyone, facilitating the diffusion and the circulation of knowledge.

Simple mapping in public health is not new. The cholera map by John Snow marked a critical turn in the use of maps to understand geographic patterns of disease [185]. Moreover, the geographic distribution of scientific data is a growing area of interest in many fields, including infectious diseases [20,186], paleontology [187], natural products research [22], microbial marine biology [188], ecology [189], and archaeology [190]. It allows the presentation of data (even old data) in new ways. For example, a paper examined the geographic origins of emerging infectious diseases from 1940 to 2004, showing non-random global patterns

Table 3

Distribution of New Delhi Metallo-beta-lactamase-1-producing bacteria reported in imported case reports by country, 1 December 2009–31 December 2012 (n=153)

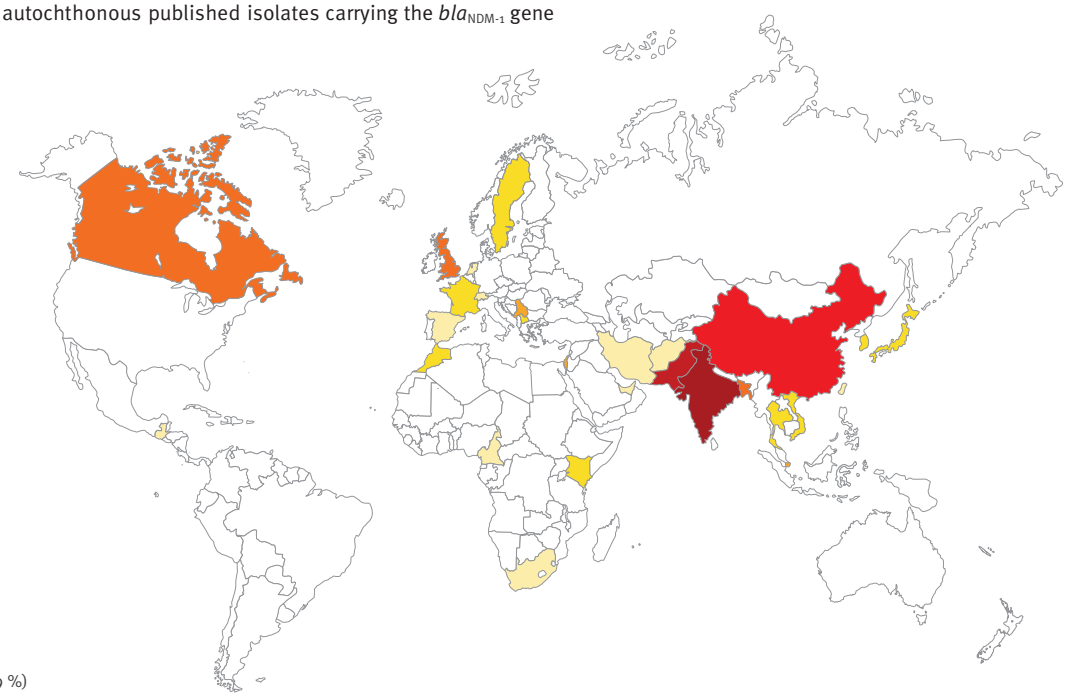
Type of case reports	Country	Cities	Imported from	Number of isolates	First description	References
Human infection	Australia	Sydney (n=3)	Bangladesh (n=1), India (n=2)	3	2010	[128-130]
	Austria	Graz (n=2)	India (n=1), Kosovo* (n=1)	2	2009-2011	[131]
	Belgium	Yvoir (n=1)	Algeria (n=1)	6	2010	[165, 196]
		Antwerp (n=2)	Montenegro (n=2)			
		Brussels (n=1)	Pakistan (n=1)			
		Namur (n=2)	Serbia and Kosovo*(n=2)			
	Canada	Brampton (n=1), Calgary (n=3), Toronto (n=1), Winnipeg (n=2)	India (n=7)	7	2010	[132-134, 162, 197]
	China	Hong Kong (n=1)	India (n=1)	1	2010	[161]
	Croatia	Zagreb (n=1)	Bosnia and Herzegovina (n=1)	1	2009	[166]
	Czech Republic	Plzeň (n=2), Prague (n=1)	Egypt (n=3)	3	2011	[170, 171]
	Denmark	Copenhagen (n=1)	Libya (n=1)	2	2011	[135, 174]
		Hvidovre (n=1)	Pakistan (n=1)			
	France	Lyon (n=3)	India (n=3)	11	2010	[57, 104, 136-138, 160, 167, 168, 172, 175]
		Marseille (n=1)	India (n=1)			
		Paris (n=5)	Algeria (n=1), India (n=1), Iraq (n=1), Serbia (n=2)			
		Saint Pierre (n=2)	India (n=1), Mauritius(n=1)			
	Germany	Not documented (n=1)	Egypt (n=1),	3	2007	[139, 164, 169]
		Bonn (n=1)	India (n=1),			
		Frankfurt (n=1)	Serbia (n=1)			
	Ireland	Dublin (n=1)	India (n=1)	1	2011	[140]
	Italy	Bologna (n=6), Siena (n=8)	India (n=14)	14	2009-2010	[141, 142]
	Japan	Niigata (n=1), Tochigi (n=1), Tokyo (n=1), Soka (n=1)	India (n=4)	4	2009	[106, 143, 144, 159]
	Kuwait	Jabriya (n=2)	India (n=2)	2	2010-2011	[145]
	Lebanon	Beirut (n=4)	Iraq (n=4)	4	2008-2011	[177, 178]
	Netherlands	Utrecht (n=2)	India (n=2)	3	2008	[120, 146]
		Enschede (n=1)	Serbia (n=1)			
	New Zealand	Porirua (n=4)	India (n=4)	4	2009-2010	[147]
	Norway	Tromsø (n=2)	India (n=2)	2	2010	[148]
	Oman	Muscat (n=14)	India (n=14)	14	2010	[149, 150]
	Singapore	Singapore (n=1)	India (n=1)	1	2010	[151]
	South Africa	Johannesburg (n=1)	Mozambique and Zambia (n=1)	1	2010	[173]
	Spain	Barcelona (n=1), Madrid (n=1)	India (n=2)	2	2011	[152, 153]
	Sweden	Örebro (n=1)	India (n=1)	1	2009	[10]
	Switzerland	Geneva (n=2)	India (n=1), Serbia (n=1)	2	2009-2010	[112, 154]
	Taiwan	Taipei (n=5)	China (n=4), India (n=1)	5	2010	[155, 179]
	Turkey	Istanbul (n=1)	Iraq (n=1)	1	2011	[176]
	United Kingdom	Bristol (n=5)	India (n=5)	44	2008	[7, 123-125]
		London(n=39)	India (n=38), Kenya (n=1)			
	United States	Atlanta (n=3)	India (n=3),	9	2010	[8, 156-158, 180]
		Chicago (n=1)	India (n=1)			
		Los Angeles (n=3)	Pakistan (n=3)			
		Providence (n=2)	Vietnam (n=1), India (n=1)			

* This designation is without prejudice to positions on status, and is in line with UNSCR 1244/99 and the ICJ Opinion on the Kosovo declaration of independence.

Figure 3

The worldwide distribution of New Delhi Metallo-beta-lactamase-1-producing bacteria 1 December 2009–31 December 2012 (n=950)

A. Worldwide distribution of autochthonous published isolates carrying the *bla*$_{NDM-1}$ gene



India (52.3 %)

Pakistan (16.7 %), China (11.9 %)

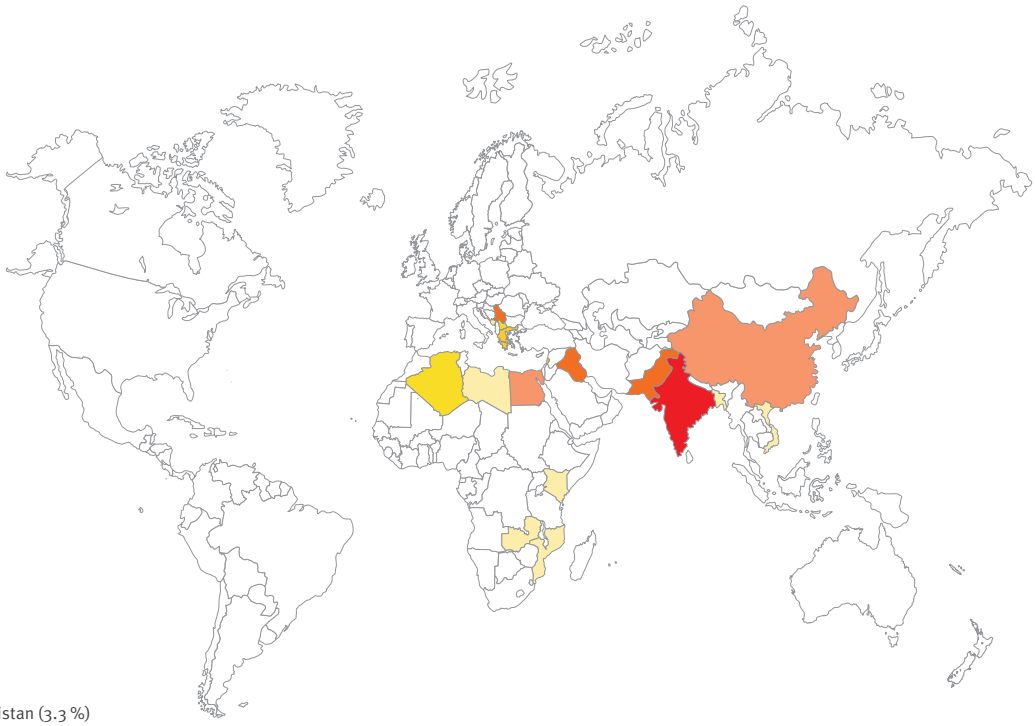
United Kingdom (2.9 %), Canada (2.3 %), Bangladesh (2.1 %)

Singapore (1.9 %), Israel (1.2 %), Serbia (1.0 %)

Kenya (0.9 %), Kosovo* (0.9 %), Thailand (0.7 %), France (0.5 %), Japan (0.5 %), Morocco (0.5 %), South Korea (0.5 %), Sweden (0.5 %), Vietnam (0.5 %)

Switzerland (0.4 %), Afghanistan (0.2 %), Guatemala (0.2 %), South Africa (0.2 %), United Arab Emirates (0.2 %), Iran (0.1 %), Mauritius (0.1 %), The Netherlands (0.1 %), Spain (0.1 %), Taiwan (0.1 %), Cameroon (0.1 %)

B. Putative countries of origin for imported published isolates carrying the *bla*$_{NDM-1}$ gene



India (75.2 %)

Serbia (3.9 %), Iraq (3.9 %), Pakistan (3.3 %)

Egypt (2.6 %), China (2.6 %)

Algeria (1.3 %), Kosovo* (1.3 %), Montenegro (1.3 %)

Bangladesh (0.6 %), Kenya (0.6 %), Bosnia and Herzegovina (0.6 %), Mozambique and Zambia (0.6 %), Vietnam (0.6 %), Libya (0.6 %), Mauritius (0.6 %)

* This designation is without prejudice to positions on status, and is in line with UNSCR 1244/99 and the ICJ Opinion on the Kosovo declaration of independence.

NDM-1: New Delhi Metallo-beta-lactamase-1

[191]. Another online, real-time disease outbreak monitoring system, 'HealthMap', developed by the John Brownstein and his team in 2008, has demonstrated the effectiveness of collecting new media sources for improved situational awareness of infectious disease worldwide [192].

Given the popularity of Google Maps, it can be expected that Google will continue to add new features, such as higher resolution, more options for the maps, three-dimensional views, and a Smartphone application. Smartphone applications are a growing field that offers novel approaches, with software that allows data entry and retrieval of data from the maps using a mobile phone [193,194]. The possibilities are vast and for all those interested to better convey information we propose to keep an open mind and test different visual representations. We strongly encourage epidemiologists to embrace new types data collection by using interactive tools for surveillance purposes and perhaps more importantly to communicate these data to other members of the research community and the general public in real time. Using detailed maps to convey such data visually helps to break down communication barriers and bring diverse research ideas together [22].

Conflict of interest

None declared.

Authors' contributions

MB, SMD, LM, PP, MD, DR, and JMR analysed the data, and wrote the manuscript. MB and SMD collect the data and build the map.

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Rotavirus vaccination coverage and adherence to recommended age among infants in Flanders (Belgium) in 2012

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In Belgium, rotavirus vaccination has been recommended and partially reimbursed since October 2006. Through a retrospective survey in 2012, we estimated the coverage rate of the rotavirus vaccination in Flanders among infants born in 2010. Using a standardised questionnaire, 874 families were interviewed at home, collecting information on demographic characteristics, socio-economic background and documented vaccination history (updated from medical files and vaccination database, if needed). Adherence to the recommended age for vaccination (8, 12 and 16 weeks) was also assessed. The coverage rate for two doses of rotavirus vaccination was 92.2% (95% confidence interval: 90.2–93.8). Respectively 31.7% and 10.1% of the children received their first and second dose at the recommended age. Incomplete vaccination was often a deliberate choice of the parents. Only eight children (1%) were vaccinated after the maximum age of 26 weeks. Factors identified by multiple logistic regression as related to incomplete vaccination were: living in the province of Antwerp, unemployed mother, and three or more older siblings in the household. Four years after introduction, the coverage rates were surprisingly high for a vaccine that is not fully reimbursed and not readily available in the vaccinator's fridge, which is the case for the other recommended infant vaccines.

Introduction

Rotavirus is the most common cause of fatal and severe childhood diarrhoea worldwide. The introduction of rotavirus vaccination into national immunisation programmes has contributed to a significant decrease in rotavirus gastroenteritis-related mortality and morbidity [1,2]. In Belgium, the national immunisation technical advisory group (NITAG) recommended rotavirus vaccination in October 2006. Unlike other infant vaccines in the national immunisation schedule, rotavirus vaccination is not offered fully free of charge by the government. If parents wish to have their child vaccinated against rotavirus, they need a prescription for the vaccine, via a well-baby clinic, general practitioner or paediatrician. Both vaccines, Rotarix (two-dose schedule) and RotaTeq (three-dose schedule), are only available in private pharmacies in Belgium. The partial reimbursement system entails that parents pay EUR 11.60 per prescribed vaccine dose, the National Health Insurance covers the remaining EUR 59.60 per Rotarix vaccine and EUR 40.10 per RotaTeq vaccine. Following the national recommendations issued by the NITAG, the first dose of rotavirus vaccine should be administered at eight weeks of age. A minimum interval of four weeks between doses should be respected and the upper age limit is set at six months (24 weeks for the monovalent Rotarix vaccine and 26 weeks for the pentavalent RotaTeq vaccine, according to the recommendations issued in 2009). Catch-up vaccination of missed doses above this age is not recommended. Concomitant administration of rotavirus vaccine with other infant immunisations is approved [3].

In 2008, vaccine coverage in children 18 to 24 months of age in Flanders was approximately 30% for two doses of the rotavirus vaccine, as measured by a survey using the World Health Organization's Expanded Programme on Immunization (EPI) methodology [4]. This low rate could be explained by the recent introduction of the vaccine in Belgium at that time. More recent coverage estimates for rotavirus vaccination were based solely on sales and reimbursement figures provided by the National Health Insurance [5].

With this study we aimed to investigate coverage rates for rotavirus vaccination among infants born in 2010 in

Flanders. We also looked at timeliness of vaccination with regard to recommended age and assessed the validity of the vaccine doses taking into account minimum and maximum age and interval parameters. Using survey-based multiple logistic regression we identified predictive factors for non-vaccination.

As Belgium was the first country in the European Union (EU) to introduce a universal rotavirus vaccine programme, these coverage estimates could contribute to the decision-making process for rotavirus vaccine introduction in other countries. Putting the results into perspective of our co-financing policy may provide insights into equitable distribution of rotavirus vaccines.

Methods

Survey Design

The methodology of the EPI-based two-stage cluster sampling design for vaccination coverage studies in Flanders has been extensively described elsewhere [6]. The sample size was calculated using the following assumptions: a minimal anticipated coverage of 90% and a design effect of 1.5. Taking into account a margin error of the confidence interval of 2.5% and a drop-out rate of 10%, this resulted in a sample size of 900 children.

A cluster random sample of toddlers (born between 1 July and 1 October 2010) was drawn from the Flemish register of natural persons. Firstly, 125 clusters proportionally distributed over the 14 districts (representing the third administrative level) of Flanders, were selected in a proportionate random way. In a second stage, seven children of eligible age were randomly selected per cluster. An overselection of 70 children in less densely populated districts was done to assure acceptably accurate estimates on coverage rates in those geographical regions. Selected families were informed by letter of a home visit by a trained interviewer. Children were replaced within the same cluster when (i) the interviewer was not able to contact the family after three home visits, of which one was after office hours, or (ii) the interviewee was not able to understand the questions because only a Dutch version of the questionnaire was available. If parents refused to participate, they were asked to state the reason for refusal, and the child was not replaced in order to reduce the risk of selection bias, as refusal could be linked with a negative attitude towards vaccination.

The visits were performed between 25 April and 7 July 2012, so all participating children should have completed their vaccination according to the schedule. Informed consent from a parent or caregiver had to be obtained for the full data collection procedure. The following information was collected through a standardised questionnaire: demographic characteristics, socio-economic background and documented vaccination history. The vaccination data available at home were checked against the Flemish immunisation registry, Vaccinnet, and completed if more information was available in that database. Thereafter, the collected data from children who were still not found to be vaccinated appropriately for their age were sent to the general physician or paediatrician (when contact information was available) with a request to verify, correct and/or complete these data.

This study was authorised by the National Privacy Commission and received approval on 16 April 2012 from the ethics committee of the Antwerp University Hospital, after consulting the ethics committee of the University of Leuven (KULeuven).

Definitions

To assess adherence to the recommended age of vaccination, we compared the vaccination history of the child with the recommended number of doses, the minimum and maximum allowed age for each dose and the minimum acceptable interval between doses. Following the national guidelines, the first rotavirus vaccine dose should be administered at the age of eight weeks, with an interval between consecutive doses of four weeks, and the last dose before the age of six months (i.e. 26 weeks). Doses that were not documented on the vaccination card, or could not be retrieved through consultation of medical files and Vaccinnet, were considered as not administered. Only the date of administration of the rotavirus vaccine was registered in the questionnaire, the brand name was not requested because this is usually not indicated in the vaccination card. Since we could not make a distinction between the two different rotavirus vaccine brands, we considered a schedule with at least two doses as complete. We defined a valid schedule as a complete schedule where all minimum and maximum age recommendations and interval parameters were strictly respected. We excluded doses that were administered more than five days before the minimum age or with an interval from the previous dose that was more than five days shorter than allowed, and doses that were administered after the age of 26 weeks. The ethnic background of parents was determined based on their country of birth as well as that of their parents (the child's grandparents): if one of them was born outside the EU (27 countries, as of 2012), the parent was categorised as non-European; if a parent or grandparent was born in the EU, but not in Belgium, the parent was categorised as European, otherwise as Belgian.

Statistical analysis

Oversampling was adjusted for by weighing if appropriate. Vaccine coverage analysis was performed using R 2.15.2 *(The R Foundation for Statistical Computing, 26-10-2012)* and presented with a 95% confidence interval (CI). We examined whether the following characteristics were related to the vaccination status at the age of 18 months: sex, main vaccinator, change of vaccinator, number of illnesses, family structure, hierarchy within the family, number of children in the family,

Table 1

Vaccination coverage at the age of 18 to 24 months per province in Flanders, 2012 (n=874)

	Antwerp[a] n=226	Limburg n=120	East Flanders n=200	Flemish Brabant n=146	West Flanders n=182
	Coverage rate (95% confidence interval)				
Rota 1*	89.8 (85.3–93.1)	98.3 (93.6–99.6)	97.0 (93.3–98.6)	94.5 (89.4–97.2)	92.9 (88.0–95.9)
Rota 2**	88.1 (83.3–91.6)	97.5 (92.3–99.2)	94.0 (89.7–96.6)	93.8 (88.5–96.8)	90.7 (85.3–94.2)

[a] For two children no documentation could be retrieved for any recommended vaccine; they were considered not vaccinated.

*p=0.013

**p=0.028

socio-economical characteristics of mother and/or father, family income, day care attendance, breastfeeding and duration of breastfeeding. Final models were selected using a backward selection, p values ‹0.05 were considered significant. Both survey-based univariate and multiple logistic regression were analysed using R 2.15.2 *(The R Foundation for Statistical Computing, 26-10-2012)*.

Results

Study population

In total, 1,064 families were contacted, including replacement contacts for 118 families (11.1%). Among the 946 families who were reached, 874 families (92.4%) participated in the study, while 72 families refused to participate (7.6%). The mean age of the children at the moment of interview was 20.9 months (age range: 18.5–23.9 months). The study population consisted of 49.9% males. All demographic characteristics, except employment status of the parents, were in line with national census estimates for these age groups. Among the participating families we found a higher proportion with both parents employed.

Coverage rate

In 92.1% of the families, a vaccination document was available at home. After additional consultation of medical files and vaccination database (Vaccinnet), 94.0% of the children between 18 and 24 months of age had proof of administration of at least one dose of rotavirus vaccine. A second dose had been administered to 92.2% of these children and 12.2% had received a third dose. A sensitivity analysis, considering as not vaccinated against rotavirus the children for whom the parents refused to participate in the coverage study (worst case scenario), resulted in a coverage decrease of 7% for each dose.

Table 1 shows a statistically significant difference in vaccination coverage between provinces, with the lowest coverage in the province of Antwerp (p=0.013 for the first and p=0.028 for the second rotavirus vaccine dose). There was also a statistically significant difference between the three districts within the Antwerp province, with the lowest coverage found in the most urbanised district, Antwerp city (Table 2).

Validity and timeliness

The so-called valid coverage rate for two doses was 90.5%. One child had received the first dose before the age of eight weeks, six children had received the second dose less than four weeks after the first, and eight other children were vaccinated after the age of 26 weeks; these doses were considered invalid.

Table 3 shows the compliance of rotavirus vaccination with the age recommendations. While 31.7% of the vaccinated children received their first dose at the recommended age, correct timing dropped to 10.1% for the second dose. In almost 30% of the children, the second dose was administered more than four weeks after the recommended age.

Assessment and parents' reasons for incomplete vaccination

At the beginning of the interview, the parents were asked to assess the vaccination status of their child (i.e. complete or not). Among the 68 children with an incomplete rotavirus vaccination schedule (excluding the 15 children who had received invalid doses outside the recommended time period), 57 parents (84%)

Table 2

Vaccination coverage at the age of 18 to 24 months per district in Antwerp, 2012 (n=226)

	Antwerp n=114	Mechelen n=53	Turnhout n=59
	Coverage rate (95% confidence interval)		
Rota 1*	83.3 (75.3–89.1)	96.2 (86.1–99.1)	96.6 (87.4–99.2)
Rota 2**	79.8 (71.5–86.2)	96.2 (86.1–99.1)	96.6 (87.4–99.2)

*p=0.003

**p=0.003

Table 3

Distribution of age at vaccination according to the guidelines of the Superior Health Council, Flanders, 2012 (n=874)

	Not	Too early	At recommended age[a]	1–3 weeks too late	4–7 weeks too late	≥ 8 weeks too late
	Number (%)					
Rota 1	52 (6.0%)	1 (0.1%)	277 (31.7%)	388 (44.4%)	119 (13.6%)	37 (4.2%)
Rota 2	68 (7.8%)	-	88 (10.1%)	457 (52.3%)	165 (18.8%)	96 (11.0%)

[a] At recommended age was defined as not before minimum age and not more than six days after the recommended age according to the guidelines (Rota 1 at eight weeks; Rota 2 at 12 weeks).

Table 4

Predictive factors for having received an incomplete schedule for rotavirus vaccination (logistic regression, univariate analysis), Flanders, 2012

		Odds ratio	95% CI
Province (n=874)	Limburg (n=120)	**0.20**	**0.06–0.67****
	East Flanders (n=200)	**0.44**	**0.21–0.91***
	Flemish Brabant (n=146)	0.50	0.23–1.10
	West Flanders (n=182)	0.79	0.41–1.52
	Baseline: Antwerp (n=226)	**1**	
Day care attendance during the first year of life (n=873)	Non-professional day care (n=75)	0.93	0.35–2.50
	Combination (professional and non-professional day care) (n=112)	0.26	0.06–1.08
	No attendance (n=165)	**2.17**	**1.23–3.85****
	Baseline: professional day care (n=521)	**1**	
Main vaccinator[a] (n=864)	Paediatrician (n=104)	1.43	0.65–3.13
	General practitioner (n=31)	**6.67**	**2.86–14.29****
	Baseline: well-baby clinic (n=729)	**1**	
Number of older siblings in the household (n=874)	None (n=76)	1.79	0.76–4.17
	One (n=304)	0.76	0.37–1.54
	Two (n=114)	1.56	0.70–3.45
	Three or more (n=49)	**5.26**	**2.38–11.11****
	Baseline: only child (n=331)	**1**	
Family income (n=755)	EUR 2,001–3,000 (n=227)	**0.38**	**0.18–0.78****
	EUR 3,001–4,000 (n=313)	**0.32**	**0.16–0.65****
	› EUR 4,000 (n=92)	**0.18**	**0.06–0.63****
	Baseline: ≤ EUR 2,000 (n=123)	**1**	
Mother's employment situation (n=869)	Part-time salary (n=213)	1.04	0.49–2.22
	Self-employed (n=27)	2.44	0.68–9.09
	Unemployed (n=211)	**3.33**	**1.89–6.25****
	Baseline: full-time salary (n=418)	**1**	
Origin of the mother (n=869)	Other EU country (n=60)	1.59	0.59–4.17
	Outside EU (n=159)	**2.94**	**1.69–5.00****
	Baseline: Belgium (n=650)	**1**	
Father's employment situation (n=819)	Part-time salary (n=16)	0.91	0.12–7.14
	Self-employed (n=92)	1.11	0.46–2.70
	Unemployed (n=51)	**4.76**	**2.33–10.00****
	Baseline: full-time salary (n=660)	**1**	
Origin of the father (n=819)	Other EU country (n=51)	2.17	0.81–5.88
	Outside EU (n=146)	**3.70**	**2.08–6.67****
	Baseline: Belgium (n=622)	**1**	
Educational level of the father (n=819)	Vocational secondary school (n=60)	0.30	0.11–1.59
	Secondary school, first cycle (n=47)	0.20	0.04–1.12
	Secondary school, second cycle (n=329)	0.36	0.13–1.04
	Bachelor degree (n=213)	**0.17**	**0.05–0.57****
	Master's degree (n=136)	**0.30**	**0.09–0.99***
	Baseline: Primary school (n=34)	**1**	

CI: confidence interval; EU: European Union.

[a] For only six children the main vaccinator was other than well-baby clinic, paediatrician or general practitioner.

*p<0.05

**p<0.01

Table 5

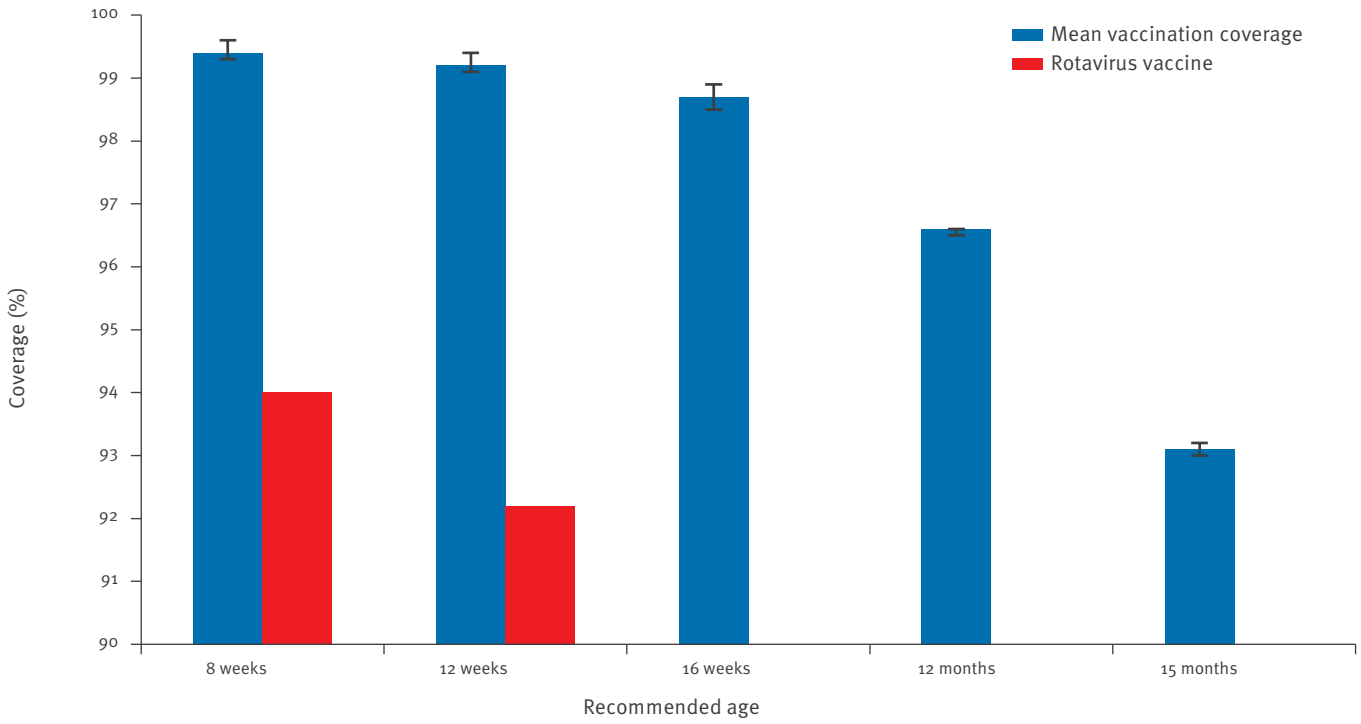
Predictive factors for having received an incomplete schedule for rotavirus vaccination (multiple logistic regression), Flanders, 2012 (n=874)

		Odds ratio	95% CI
Province (n=874)	Limburg (n=120)	**0.21**	**0.06–0.72***
	East Flanders (n=200)	**0.45**	**0.21–0.95***
	Flemish Brabant (n=146)	0.49	0.22–1.11
	West Flanders (n=182)	0.85	0.43–1.67
	Baseline: Antwerp (n=226)	**1**	
Rank in the household (n=874)	First child (n=76)	1.61	0.65–4.00
	Second child (n=304)	0.76	0.38–1.54
	Third child (n=113)	1.25	0.53–2.94
	Fourth child or younger (n=50)	**4.00**	**1.72–10.00****
	Baseline: only child (n=331)	**1**	
Mother's employment situation (n=869)	Part-time salary (n=213)	0.87	0.40–2.22
	Self-employed (n=27)	2.04	0.60–7.14
	Unemployed (n=211)	**2.56**	**1.35–5.00****
	Baseline: full-time salary (n=418)	**1**	

CI: confidence interval.
*p<0.05
**p<0.01

Figure

Comparison of vaccination coverage at the recommended age of rotavirus vaccine and vaccines offered free of charge, in children aged 18–24 months, Flanders, 2012 (n=874)



Note: Y-axis does not start at zero

were convinced that their child had received all the recommended vaccines, while two parents had no idea whether the vaccination status was complete or not.

After consulting the vaccination card, parents of the children with missing rotavirus vaccine doses were asked for reasons of incomplete vaccination. At this point, 22 parents (32%) still assumed that all doses had been administered but not documented, 18 parents (27%) could not think of any reason why the rotavirus vaccination schedule was incomplete, and 27 (40%) were aware of their child being unvaccinated; among the latter, the majority (n=18) chose deliberately not to do vaccinate their child. Reasons mentioned by the remainder included illness and missed appointments. Age restrictions for initiating and completing the rotavirus vaccination schedule were mentioned only once.

Factors related to vaccination status

Univariate analysis

The univariate analysis identified the following factors as significantly related to a lower probability of being fully vaccinated against rotavirus: living in the province of Antwerp, not attending day care during the first year of life, general practitioner as main vaccinator, three or more older siblings in the household, unemployed mother and/or father, and origin of mother and/or father outside the EU (Table 4). A monthly income higher than EUR 2,000 resulted in a higher probability of being fully vaccinated, as did a higher educational level of the father. Breastfeeding and educational level of the mother were not related to vaccination status.

Multivariate analysis

Since many of these characteristics are related, we examined these influencing factors through a multivariate approach. In view of the relation between maternal and paternal factors, we decided to retain only the maternal parameters. Previous coverage studies have shown that many influencing factors were related to the main vaccinator. Therefore, it was decided to exclude the latter.

The following factors were identified in a multiple regression analysis as being related to incomplete vaccination: living in the province of Antwerp, unemployed mother, and three or more older siblings in the household (Table 5).

Discussion

Since Belgium adopted the rotavirus vaccination early, using a co-financing policy, it represents an interesting opportunity to assess rotavirus vaccine uptake comparing with other recommended infant vaccines, free of charge. This study falls within the ambit of the 2011 conclusion of the Council of the European Union on childhood immunisation which emphasised the need for high quality national data on vaccine coverage rates [7,8].

The coverage rate for rotavirus vaccination for the infant population in Flanders born in 2010 exceeded 90%, four years after the introduction of the vaccine on the Belgian market and publication of the recommendation. This is a major increase compared with the coverage of 30% (for two doses of rotavirus vaccine) registered among children born in 2006, shortly after introduction of the rotavirus vaccine [4]. The pentavalent rotavirus vaccine was licensed and recommended for routine immunisation of infants in the United States (US) in February 2006, a couple of months before Belgium [9]. National coverage estimates on rotavirus vaccination coverage in the US were reported for the first time in the 2009 National Immunization Survey (NIS): 43.9% of the children born within two years of licensure had full coverage [10]. The most recent NIS in the US reported an increase in rotavirus vaccination coverage from 59.2% in 2010 to 68.6% in 2012 [11].

A coverage rate of 92.2% is very high for a vaccine for which the parents have to co-pay per administered dose, but still lower than the coverage (93–99%) achieved for other infant vaccines recommended in the National Immunization Programme (NIP) for the first year of life and offered free of charge by the government (Figure).

Besides rotavirus vaccination, the survey also covered other childhood immunisations included in the infant immunisation schedule in Flanders [12] for which we examined possible predictive factors for undervaccination. The observed differences between provinces in Flanders were only significant for rotavirus vaccination and not for other infant immunisations. Further looking into the Antwerp province revealed that the most urbanised district had the lowest coverage rates for rotavirus vaccination. This might indicate a risk factor related to urbanisation that could not be explored thoroughly using the characteristics that were collected during the interview.

The negative impact of a large number of older siblings on rotavirus vaccine uptake may partly be explained by the fact that rotavirus vaccines were not yet available at the time the older siblings received their infant immunisations, leading to decreased awareness among these parents. Logistical problems associated with large families may also have contributed to the lower vaccination coverage. Unemployment status of the mother has previously been identified as one of the socioeconomic factors related to an incomplete vaccination status for other infant vaccines in Belgium [6,16]. In the current survey, this was the case not only for the rotavirus vaccine but also for infant doses of the pneumococcal and measles-mumps-rubella vaccine [12]; there is therefore no solid evidence to argue that this could be related to the fact that parents have to co-pay for rotavirus vaccines.

Although the multivariate analysis did not identify any inequality that may have been introduced by the

co-payment system, one could question whether this is the most efficient way in terms of budget allocation. It has been calculated that fully funded universal vaccination would be at least 30% less expensive (if the coverage is 80%) and more cost-effective compared to the current situation, if a well negotiated vaccine price were achieved, e.g. through a tender system [17].

It is of utmost importance to complete the vaccination schedule once it is initiated. Only 2% of the children that received a first dose of rotavirus vaccine did not complete their schedule. The proportion of children who completed their vaccination series in Flanders is somewhat larger than the reported proportion based on US health insurance claims for under one year-olds, where 9% of the children immunised with the monovalent vaccine missed a final dose, and 16.6% of the children who received a first dose of the pentavalent vaccine did not complete their schedule [14]. In our study it was not possible to distinguish between vaccine brands; therefore we might have misinterpreted the completeness of the vaccination schedule, since two doses (considered as fully vaccinated in our study) might have been an incomplete vaccination schedule with Rotateq (needing three doses to be complete). However, the two-dose monovalent vaccine Rotarix has the highest market share in Belgium (>85%) [15]. A third dose was documented in 109 children (12.5%; data not shown) [12], which is in line with the market share of the pentavalent vaccine. Still, the possible overestimation of the completeness of the schedules may have led to an overestimation of the coverage.

If parents were aware that a rotavirus vaccine dose had been missed, the omission seemed to be a deliberate choice (based on the small number of parents in our sample). Further investigation is needed into whether this was related to the cost of the vaccine, to the low perceived risk of the disease or to practical barriers. However, the multiple regression analysis that corrected for several socio-economic characteristics found no association between an incomplete schedule for rotavirus vaccination and income of the family. For those children who did not complete their initiated schedule, illness was most frequently mentioned as a reason.

There is a decreasing trend in coverage with advancing recommended age for all infant vaccine doses including rotavirus vaccine (Figure) [12]. However, the latter has a lower coverage than the other infant vaccines at any time point, which could be due to the fact that rotavirus vaccines are not available free of charge at the vaccinators' sites; parents first need a prescription from the physician to purchase the vaccine at the pharmacy, so they may not have the vaccine with them when they take the child for administration of the other vaccines [12].

A recent study in Australia showed that the introduction of RotaTeq into the national immunisation programme increased the timeliness of the uptake of the third dose of diphtheria, pertussis and tetanus (DTP)-containing vaccine due to the strict dosing schedule of the rotavirus vaccine [18]. We did observe a positive trend between 2008 and 2012 in the timely administration of the DTP-containing vaccines in the first year of life [12,19]. This cannot be conclusively attributed to the influence of introducing the rotavirus vaccine, since timeliness has been a major topic in many campaigns in well-baby clinics, the main vaccinator in Flanders.

Considering the observed timeliness of rotavirus vaccination, compliance with the recommendations could be improved. The purpose of the recommended vaccination schedule is to protect every child as soon as possible and to minimise the period in which they are prone to infections. Any delay in vaccination can have a major impact, especially for diseases like rotavirus where multiple vaccine doses are required for protection [20] and disease risk in infancy is considerably high [21]. The majority of the vaccinated children received their second dose too late, although only eight children were vaccinated after the age of 26 weeks. The adherence to this upper age limit is of importance in view of the recent postmarketing surveillance data on the small increase in risk of intussusception (1–2/100,000 vaccinated infants) shortly after the first dose [22]. Considering the increased background rates of intussusception in older infants [23], catch-up vaccination is not recommended.

It is recommended to respect the minimum interval of four weeks between consecutive doses in order not to compromise the efficacy of the administered doses, otherwise this results in the necessity to re-administer the dose. Only six children received a second or third dose without respecting the recommended four-week interval between doses.

A selection bias may have occurred in this study due to a possible correlation between vaccination status and willingness to participate in this study. Although the refusal rate was low (7.6%), we cannot exclude overestimation of the coverage rates. It is unlikely that all parents who refused to participate in the study would have refused rotavirus vaccination for their child. But even if this were the case, the coverage rate would be acceptable at 85%. On the other hand, taking into consideration only documented vaccination history may have led to an underestimation, although every effort was made to obtain documented vaccination history.

Conclusion

The effectiveness of the implementation of rotavirus vaccination in Belgium has previously been demonstrated by the tremendous impact on the number of hospitalisations, with a reduction of 33% in the number of hospital admissions due to acute gastroenteritis during 2007–09, and on the number of laboratory-diagnosed cases, with a decrease of 61.4% in 2008 compared with the pre-vaccination period [5,15,24]. This

public health benefit could only be achieved because of the good performance of the rotavirus vaccination [15] in combination with a high coverage, although recent studies in Europe demonstrated that even a low rotavirus vaccine uptake may have significant effects on the disease burden [25,26].

Our results suggest that further efforts are necessary to identify those children that are not reached through the current vaccination strategies. Another issue for improvement is the timeliness of rotavirus vaccination. This was also emphasised by the World Health Organization in their most recent position paper, which calls for efforts to ensure the simultaneous administration of rotavirus vaccine with DTP-containing vaccines in a timely manner, in order to induce protection before natural rotavirus infection [22].

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Conflict of interest

NH: partly funded by Scientific Chair in Evidence-based Vaccinology sponsored by hand gift, Pfizer (2009-2014). PVD and KH: principal investigator of vaccine trials for several vaccine manufacturers.

Authors' contributions

TB, HT, TL, MR, KH, PVD were all involved in the design, coordination and data management of the study of vaccine coverage in Flanders in 2012, that covered different age groups. NH performed the logistic regression analysis

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A low-cost, sustainable, second generation system for surveillance of people living with HIV in Spain: 10-year trends in behavioural and clinical indicators, 2002 to 2011

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A second-generation surveillance system of people infected with human immunodeficiency virus (HIV) has been implemented in Spain. Behavioural and clinical data were collected between 2002 and 2011 through an annual one-day, cross-sectional survey in public hospitals, including all in- and outpatients receiving HIV-related care on the survey day. Mean age increased over time (from 38.7 years in 2002 to 43.8 years in 2011) and 68.4% of the 7,205 subjects were male. The proportion of migrants increased from 6.1% to 15.9%, while people who inject or used to inject drugs (PWID and Ex-PWID) decreased and men who have sex with men (MSM) and heterosexuals increased. Unprotected intercourse at last sex increased among MSM and PWID/Ex-PWID. Patients receiving antiretroviral treatment increased significantly from 76.0% to

88.2% as did those with CD4 T-cell counts ≥350 (from 48.2% to 66.9%) and viral copies <200 (from 47.0% to 85.2%). HIV-infected people with hepatitis C virus RNA decreased from 36.0% in 2004 to 29.9% in 2011, while those with HBsAg remained stable at around 4.4%. Implementation of a low-cost, sustainable system for second-generation surveillance in people living with HIV is feasible. In Spain, the information obtained has helped to define and refine public health policy and document treatment effectiveness.

Introduction

Second generation surveillance for human immunodeficiency virus (HIV) is the systematic, ongoing collection of biomedical and behavioural information in all groups of interest for HIV, with the objective of monitoring changes in risk behaviour and other factors that influence the occurrence of HIV infection [1]. Implementation of second generation surveillance in people living with HIV (PLWH) is important because, if they engage in risk behaviours, they can transmit the infection to others and expose themselves to re-infection with HIV or infection with other pathogens.

Ecological studies have shown an inverse relation between uptake of HIV treatment by PLWH and new HIV diagnoses [2,3], and early treatment has recently been proven to be a highly efficacious measure to prevent HIV transmission to HIV-negative sexual partners [4]. However, even in high-income countries, linkage of HIV-positive individuals to treatment and viral load suppression is not easy to achieve [5,6]. This underlines the importance of obtaining data on treatment access and effectiveness in PLWH in order to document progress towards the control of the HIV epidemic.

In 2009, the European Centre for Disease Prevention and Control (ECDC) commissioned a mapping study of behavioural surveillance activities in European Union/European Economic Area (EU/EEA) countries. Despite the fact that PLWH are the major group of interest for the prevention and control of further spread of HIV, the results showed that behavioural surveillance activities were very scarce among this group and pointed out the challenges in collecting this type of information in a sustainable way due to difficulties in sampling this population and following up over time. In addition, this report suggested relevant indicators to be collected for PLWH which included not only behavioural information but also clinical data such as viral load or CD4 T-cell count [7].

In 2011, between 130,000 and 160,000 people in Spain were estimated to be living with HIV, around 30% of whom did not know their HIV status, and more than 90,000 were reported to be receiving highly active anti-retroviral therapy (HAART) [8,9].

Starting in 1996, when HAART was scaled up in the country, it was decided to set up a population-based information system, the 'Encuesta hospitalaria de pacientes infectados con VIH (EH)' (Hospital survey of patients infected with HIV), with the objective of obtaining up-to-date clinical and epidemiological information on PLWH. This survey has since been carried out yearly, except on two occasions, and a report of its results is published to inform clinical and public health practice [10]. Over time, the EH has been modified to include other information of interest, and in 2002, questions on sexual and other risk behaviours were introduced.

The EH is the only population-based source of clinical and behavioural information that we are aware of in the European setting which has allowed the systematic collection of information on PLWH over time. Here we present trend data from 2002 to 2011 on key behavioural and clinical indicators among PLWH in Spain.

Methods

The EH is an annual one-day cross-sectional survey that collects socio-demographic, epidemiological, behavioural, clinical and preventive measures such as vaccinations on all PLWH attending general public hospitals for HIV-related care on the day of the survey; both inpatients and outpatients are included. Because of limited resources, HIV-infected people who receive care at the hospital for reasons other than HIV are excluded.

Since a sampling frame for PLWH does not exist, the study was performed in public hospitals. The rationale behind this was that HIV care in Spain is hospital-based and free of charge, and all HIV-infected persons living in the catchment area of a public hospital receive care at that hospital; catchment areas are geographically defined and the population covered by a particular hospital is known, thus allowing coverage calculations; 'population coverage' is defined as the proportion of the total population living in a particular region/s included in the participating hospitals´ catchment

Table 1

Hospitals participating in the annual one-day cross-sectional survey, Spain, 2002–2011

Year	Hospitals providing care for HIV in the study area[a]	Participating hospitals n (%)
2002	111	79 (71)
2003	120	86 (72)
2004	112	77 (69)
2006	115	67 (58)
2007	106	77 (73)
2008	125	81 (65)
2009	127	87 (69)
2010	125	88 (70)
2011	124	91 (73)

HIV: human immunodeficiency virus.

[a] Participating regions varied from 13 in 2007 and 2011 to 16 in 2003 (of a total of 19).

Table 2

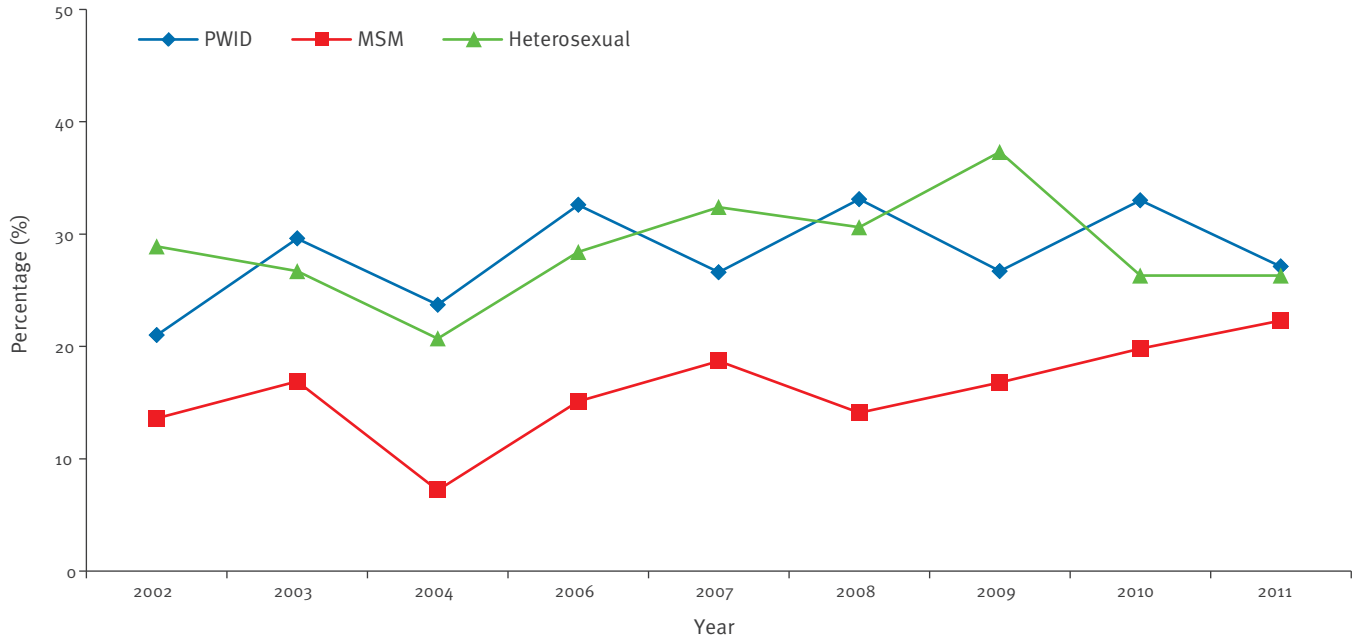
Socio-demographic and epidemiological characteristics of HIV-infected patients in Spain, 2002–2011 (n=7,205)

	2002		2003		2004		2006		2007		2008		2009		2010		2011		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sex																				
Male	663	67.8	639	65.5	577	70.4	537	65.4	485	71.0	444	68.0	530	69.7	489	69.7	562	69.2	4,926	68.4
Female	255	26.1	282	28.9	209	25.5	237	28.9	165	24.2	186	28.5	199	26.2	183	26.1	243	29.9	1,959	27.2
Unknown	60	6.1	55	5.6	34	4.1	47	5.7	33	4.8	23	3.5	31	4.1	30	4.3	7	0.9	320	4.4
Age group (years)																				
<30	92	9.4	81	8.3	46	5.6	48	5.8	40	5.9	52	8.0	57	7.5	44	6.3	53	6.5	513	7.1
30–34	183	18.7	165	16.9	118	14.4	87	10.6	74	10.8	61	9.3	68	8.9	57	8.1	62	7.6	875	12.1
35–39	292	29.9	295	30.2	205	25.0	198	24.1	141	20.6	111	17.0	134	17.6	115	16.4	115	14.2	1,606	22.3
40–49	312	31.9	349	35.8	332	40.5	367	44.7	320	46.9	313	47.9	346	45.5	325	46.3	383	47.2	3,047	42.3
≥50	92	9.4	76	7.8	106	12.9	106	12.9	96	14.1	111	17.0	146	19.2	157	22.8	189	23.3	1,079	15.0
Unknown	7	0.7	10	1.0	13	1.6	15	1.8	12	1.8	5	0.8	9	1.2	4	0.6	10	1.2	85	1.2
Country of birth																				
Spain	918	93.9	898	92.0	758	92.4	754	91.8	602	88.1	590	90.4	662	87.1	603	85.9	672	82.8	6,457	89.6
Other	60	6.1	78	8.0	62	7.6	67	8.2	81	11.9	63	9.6	98	12.9	99	14.1	129	15.9	737	10.2
Unknown	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	11	1.4	11	0.2
Transmission route																				
Heterosexual	223	22.8	236	24.2	218	26.6	208	25.3	181	26.5	185	28.3	218	28.7	217	30.9	227	28.0	1,913	26.6
MSM	159	16.3	118	12.1	129	15.7	125	15.2	137	20.1	120	18.4	171	22.5	141	20.1	199	24.5	1,299	18.0
PWID	535	54.7	546	55.9	419	51.1	444	54.1	337	49.3	305	46.7	327	43.0	302	43.0	332	40.9	3,547	49.2
Other/unknown	61	6.2	76	7.8	54	6.6	44	5.4	28	4.1	43	6.6	44	5.8	42	6.0	54	6.7	446	6.2
Employment status																				
Employed	418	42.7	443	45.4	371	45.2	367	44.7	364	53.3	311	47.6	357	47.0	295	42.0	371	45.7	3,297	45.8
Unemployed	250	25.6	241	24.7	175	21.3	184	22.4	103	15.1	133	20.4	175	23.0	172	24.5	180	22.2	1,613	22.4
Retired/disabled	210	21.5	188	19.3	194	23.7	184	22.4	159	23.3	149	22.8	156	20.5	174	24.8	205	25.2	1,619	22.5
Housewife/student	48	4.9	50	5.1	37	4.5	33	4.0	28	4.1	30	4.6	34	4.5	36	5.1	24	3.0	320	4.4
Other/unknown	52	5.3	54	5.5	43	5.2	53	6.4	29	4.2	30	4.5	38	5.0	25	2.6	32	3.9	356	4.9
Residence																				
Living alone	128	13.1	116	11.9	135	16.5	113	13.8	117	17.1	115	17.6	125	16.4	119	17.0	156	19.2	1,124	15.6
Living with family	649	66.4	733	75.1	606	73.9	583	71.0	484	70.9	479	73.4	544	71.6	508	72.4	534	65.8	5,120	71.1
Prisons	24	2.5	40	4.1	28	3.4	49	6.0	11	1.6	10	1.5	16	2.1	22	3.1	16	2.0	216	3.0
Closed institutions	46	4.7	55	5.6	30	3.7	43	5.2	47	6.9	28	4.3	47	6.2	36	5.1	29	3.6	361	5.0
Homeless	21	2.1	18	1.8	14	1.7	19	2.3	16	2.3	7	1.1	9	1.2	11	1.6	12	1.5	127	1.8
Other/unknown	110	11.2	14	1.4	7	0.9	14	1.7	8	1.2	14	2.1	19	2.5	6	0.9	65	8.0	257	3.6
TOTAL	**978**	**100.0**	**976**	**100.0**	**820**	**100.0**	**821**	**100.0**	**683**	**100.0**	**653**	**100.0**	**760**	**100.0**	**702**	**100.0**	**812**	**100.0**	**7,205**	**100.0**

HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

Figure 1

Unprotected intercourse of HIV-infected patients at last sexual encounter by transmission category, Spain, 2002–2011 (n=4,132)



HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

area. Furthermore, with a few exceptions, antiretroviral drugs are available only in public hospitals, so that the vast majority (more than 95%) of HIV-infected patients in Spain receive HIV care and treatment in public hospitals.

Participation in the survey is voluntary for both hospitals and individual patients. Between 2002 and 2011 the number of participating hospitals varied from 67 in 2006 (population coverage: 61.3% of the total population in the participating regions) to 91 in 2011 (population coverage: 72.1%), while the total hospitals providing care for HIV patients in the study area ranged from 106 in 2007 to 127 in 2009. The number of participating regions ranged from 13 in 2007 and 2011 to 16 in 2003 (of a total of 19) (Table 1).

In the 2011 survey round, 91 hospitals participated, with an overall population coverage in the participating regions of 72.1% (ranging from 36% in the region Madrid to 100% in the regions Cantabria, Castilla y León, Valencia, Navarra, La Rioja and Melilla). With regard to the whole Spanish population, survey coverage was 38.1%.

Information on the variables of interest was collected in a standard questionnaire by inpatient and outpatient medical staff. All information was extracted from the clinical records, except socio-demographic and behavioural data, which were obtained directly from the patients by the attending physician. The respondent rate varied from a minimum of 86% in 2009 to a maximum of 93% in 2010.

Most questions remained the same over the years, but some were excluded, added or modified according to what was deemed necessary at any given time. Once completed, the questionnaires were sent to the National Centre of Epidemiology, where the data were entered into a database and analysed using existing staff resources.

For continuous variables, the mean and its standard deviation were calculated and the t-test was used for comparisons. For categorical variables, distributions were calculated and the chi-squared test was used for comparisons.

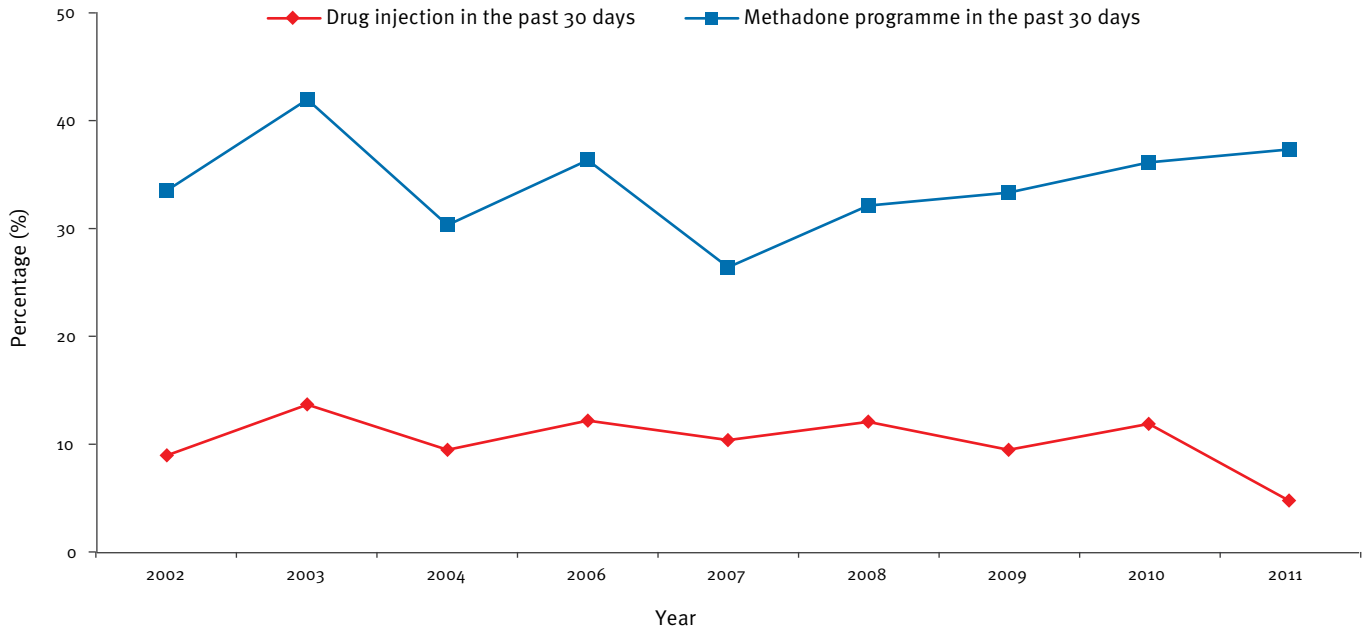
The study was performed in accordance with the requirements of the Spanish legislation on data protection. Throughout all study procedures, questionnaires were totally anonymous, i.e. no personal identifiers were collected and linkage of questionnaires to individual patients is not possible.

Results

From 2002 to 2011 a total of 7,205 PLWH were included in the study, and the majority of them were in ambulatory care, with a proportion increasing from 75.8% in 2002 to 82.8% in 2011. Respondents were predominantly male (between 65.4% and 71.0% depending on the year), and their mean age increased steadily over

Figure 2

Drug injection and participation in a methadone maintenance programme in the 30 days before the survey among current and ex-injecting drug users living with HIV, Spain, 2002–2011 (n=3,547)



HIV: human immunodeficiency virus.

time, both overall (from 38.7 in 2002 to 43.8 years in 2011) and in all transmission categories, except men who have sex with men (MSM) whose mean age increased until 2006 (from 41.4 to 44.8 years) and remained stable thereafter. The proportion of people born outside Spain also increased significantly over time from 6.1% in 2002 to 15.9% in 2011 ($p<0.05$); in the same period, the proportion of foreign-born among the total Spanish population increased from 6.2% to 14.2% [11]. With regard to transmission categories, there has been a clear decrease in the proportion of people infected through injecting drug use while the proportions of MSM and those infected through heterosexual sex have increased. Throughout the study, employment among respondents was low (45.8%), a significant proportion of patients were living in prison or other closed institutions (3% and 5% respectively) and almost 2% were homeless (Table 2).

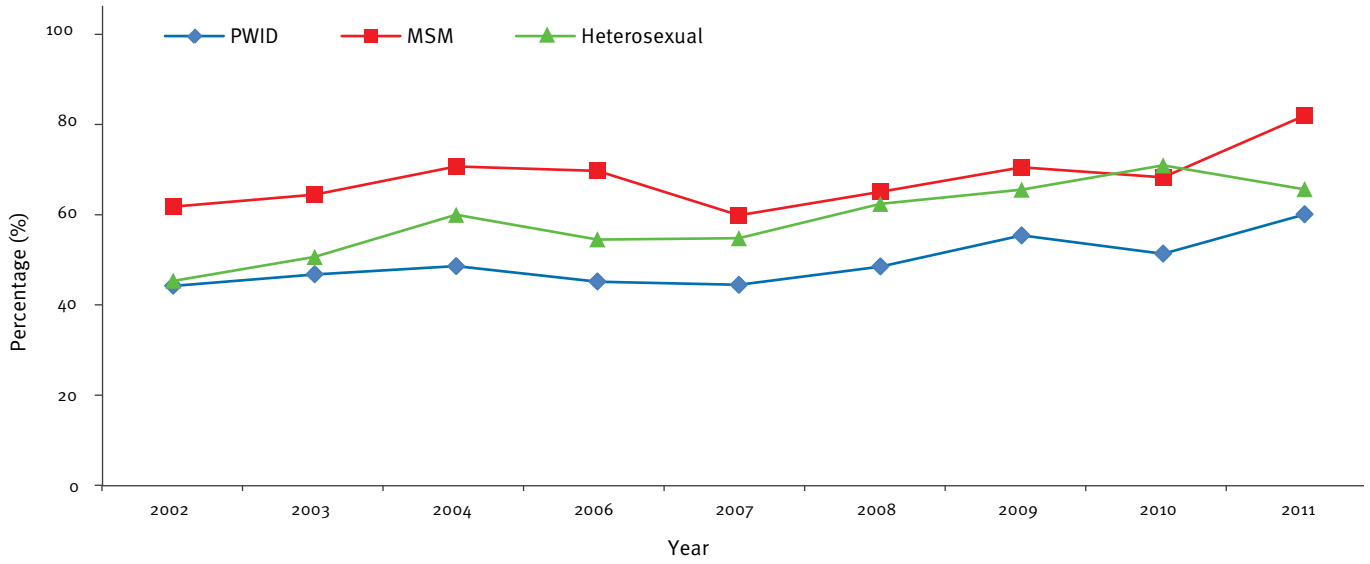
Overall, six of 10 individuals reported having had sex in the 12 months before the survey, and there was a statistically significant increase in the proportion of people who were sexually active in the age groups between 30 and 34 years (from 63.4% in 2002 to 85.5% in 2011) and between 35 and 39 years (from 64.4 in 2002 to 69.6% in 2012). By sex, there were no differences in the proportion of those reporting sexual intercourse (60.6% in men vs 62.4% in women) and no changes were obvious during the period. Overall, 62.5% of heterosexuals and 64.9% of MSM reported sexual intercourse in the past 12 months. There was a significant decrease in sexual activity among patients infected through the sharing of injecting equipment (from 60.1% in 2002 to 55.2% in 2011; $p<0.05$). No trend was observed in other risk groups.

Among individuals reporting sex in the 12 months before the survey, the global proportion reporting unprotected intercourse at last sexual encounter has remained stable over time (range: 19.6%–28.6%). However, there has been a significant increase ($p<0.05$) in unprotected sex at last sexual encounter among individuals aged between 35 and 39 years (from 17.0% in 2002 to 28.7% 2011) and between 40 and 49 years (from 17.7% to 24.6%). Similar trends were observed in MSM (from 13.6% in 2002 to 22.3% in 2011) and PWID (from 21.0% in 2002 to 27.1% in 2011). It is worth noting that although the proportion of unprotected sex among MSM increased, it was still less frequent than in the other two main transmission groups (heterosexual and PWID) (Figure 1).

Since 2011, information has been collected on condom use at last sexual encounter by HIV status of the sexual partner: of 328 patients who, in the previous 12 months, always had sex with partners of serodiscordant/unknown status, 21% did not use a condom at last sexual encounter, while the corresponding figure among the 112 reporting sex always with HIV-positive partners was 37.5%. Of the remaining 13 subjects who had sex with partners of any status, six did not use a condom at last sexual encounter.

Figure 3

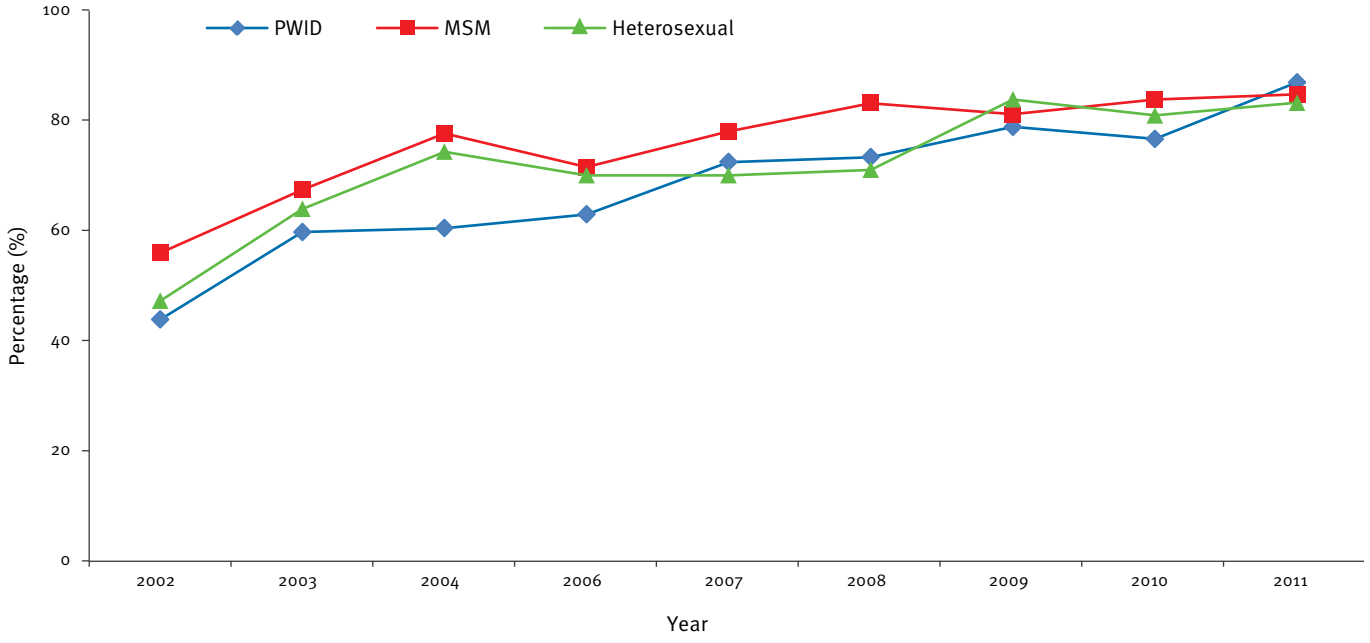
Proportion of HIV-infected people presenting more than 350 CD4 T-cells/mL at last measurement, by transmission category, Spain, 2002–2011 (n=6,759)



HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

Figure 4

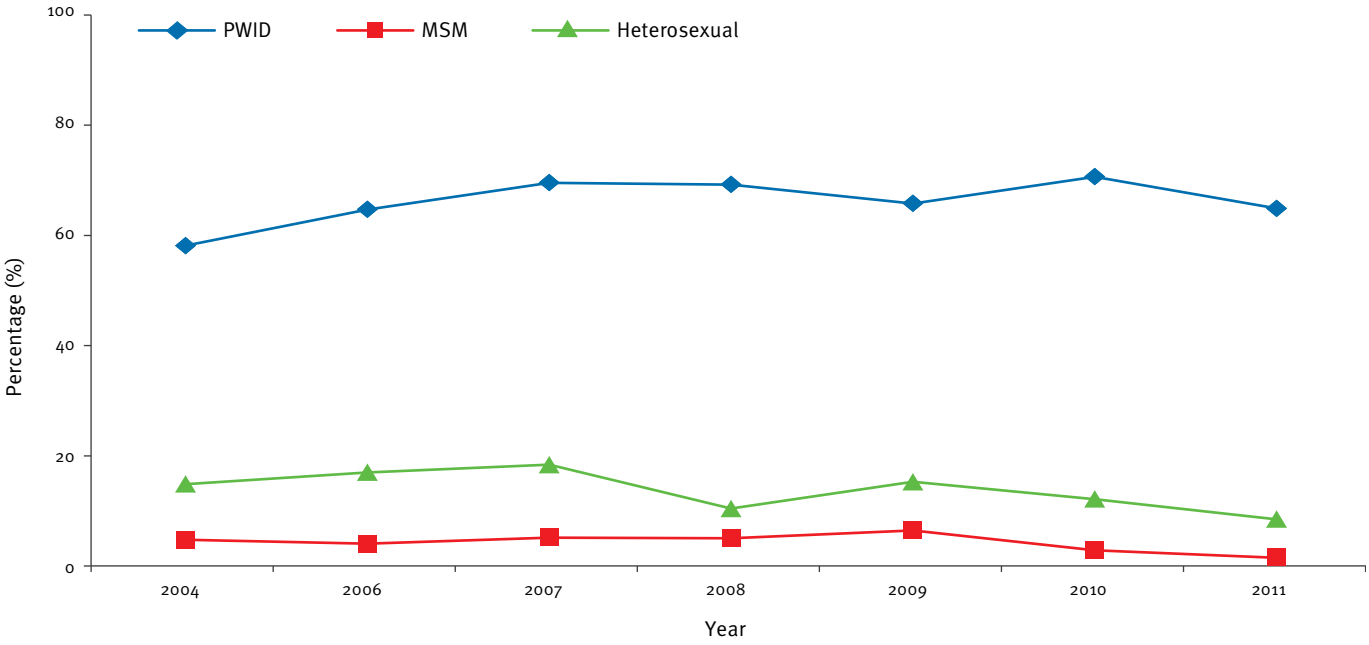
Proportion of HIV-infected people on antiretroviral treatment with undetectable viral load (<200 copies/mL) at last measurement, by transmission category, Spain, 2002–2011 (n=5,364)



HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

Figure 5

HIV-infected patients positive for HCV RNA, by transmission category, Spain, 2004–2011 (n=4,942)



HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

In 2011, 10.5% of patients reported having been diagnosed with another sexually transmitted infection in the past 12 months, generally syphilis (65%). Syphilis diagnoses increased from 0.8% in 2002 to 6.8% in 2011 ($p<0.05$), and the same trend was observed for gonorrhoea (from 0.1% to 1.8%, $p<0.05$).

In patients who acquired the infection through shared injecting equipment, data were collected on drug injection and participation in a methadone maintenance programme (MMP) in the 30 days before the survey. The proportions of respondents reporting drug injection and participation in MMPs both oscillated during the study period, but the changes were not statistically significant: drug injection ranged between a high of 13.7% in 2003 and a low of 4.8% in 2011, while MMP participation ranged between a high of 41.9% in 2003 and a low of 26.4% in 2007 (Figure 2).

Overall, 88.2% of patients in 2011 were receiving antiretroviral treatment (ART) at the time of the survey, compared with 76.0% in 2002 ($p<0.05$). There were no significant differences in the proportion of respondents on ART by transmission category, and trends followed the same increasing direction as the overall trend. Of the 91 patients who were not on treatment in 2011, 43 (47.3%) did not meet the clinical criteria [12] to receive it, 37 (40.7%) did meet them, and 11 (12.1%) had test results pending. Of the 37 patients who had a treatment indication but were not taking ART, 22 did so following a personal decision, five had interrupted ART because of toxicity, and in the remaining 10 cases treatment had just been prescribed, they were waiting to recover from another disease before initiating ART or there was no information. Among the 716 PLWH who were in treatment in 2011, 560 (78.2%) had optimal adherence according to their physician's judgment.

The most common regime (290/716; 40.5%) prescribed in 2011 comprised two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor, and the second most common (277/716; 38.7%) consisted of two NRTI and one protease inhibitor.

Between 2002 and 2011, PLWH included in the survey experienced a clear improvement in their health, as reflected by different clinical parameters: the proportion ever diagnosed with acquired immunodeficiency syndrome (AIDS) decreased from 50.9% to 37.2% ($p<0.05$); the proportion with a CD4 T-cell count greater than 349 cells at last measurement increased from 48.2% to 66.9% ($p<0.05$), and similar trends were observed in the three main transmission categories (Figure 3). The proportion of people on ART with suppressed viral load (less than 200 copies) at last measurement increased from 47.0% in 2002 to 85.2% in 2011 ($p<0.05$) (Figure 4).

The most prevalent co-morbidities in PLWH in the Spanish setting were viral hepatitides, in particular hepatitis B and C. Data collection on hepatitis B and C in the EH began in 2004. In the period from 2004 to 2011, the percentage of persons positive for HBsAg remained stable (around 4.4%), while the percentage of people positive for HCV RNA decreased significantly from 36.0% to 29.9%; however, when analysing trends by transmission categories, the decrease was observed in heterosexuals but not in PWID or MSM (Figure 5).

Apart from hepatitis, other co-morbidities were evident. In 2011, the most common were: infectious diseases (n=33, 4.1%), mental disorders (n=21, 2.6%), respiratory disorders (n=11, 1.3%) and solid neoplasias (n=9, 1.1%). Lipoatrophy was present in 188 of 812 patients (23.2%), a higher proportion, although not statistically significant, than the 158 of 820 (19.3%) notified in 2004 when this information was first collected.

Discussion

This analysis provides population-based information on PLWH in Spain and presents 10-year trend data for key behavioural and clinical indicators. Our experience with the EH indicates that it is feasible to systematically perform second generation surveillance on PLWH at low cost and in a sustainable manner. The data collected are useful for identifying changes in epidemiological patterns, for investigating risk behaviours, and for assessing treatment effectiveness and the possible impact of treatment as prevention [4].

The population infected with HIV in Spain has experienced important changes since the 1980s and 1990s with regard to main route of infection and country of origin. Sexual transmission, rather than parenteral transmission through shared injecting equipment, is now the main route of HIV transmission. The proportion of PLWH born outside Spain has also increased sharply. These changes, similar to those revealed in other information systems [13], reflect the impact of measures implemented in response to drug injection in the country [14] and the effect of migration patterns in recent years.

In our study, between 25% and 30% of PLWH who had had sex in the 12 months before the survey reported unprotected intercourse in their last sexual encounter. While the overall figure has not changed significantly during the last 10 years, the proportion reporting unprotected sex at last sexual encounter appears to be increasing among PWID and MSM. MSM are overrepresented among new HIV diagnoses in Spain and are the only transmission route with an increasing trend. MSM are also overrepresented in syphilis and gonorrhoea diagnoses performed in a network of STI clinics [15], and outbreaks of hepatitis A, syphilis and lymphogranuloma venereum [16-18] have been described among MSM in recent years. All these facts are in line with our findings and underscore the importance of reinforcing preventive measures in this group.

It is well known that HIV-positive PWID in Spain have poorer adherence to treatment than other HIV-infected patients [19]. In this context, not using condoms could be an additional sign of poorer treatment results, since the same factors of social disadvantage, lack of support and stigma that make PWID less adherent to treatment, could also make them less compliant with preventive measures recommended by their physicians, such as condom use that prevents re-infection with HIV and/or infection with other agents.

Almost 90% of PLWH in Spain were on ART in 2011, and although the proportion on treatment has always been very high, it has increased further during the last decade. This is likely to be a reflection of the less stringent international criteria to initiate treatment since HIV diagnosis and treatment have during the entire study period been available to anyone in need of it and provided free of charge.

Continued clinical improvement after HAART implementation has been reported in HIV-infected cohorts in Spain [20-22]. Our results confirm these findings and reflect very good treatment effectiveness, confirming that ART is an effective prevention tool. Unfortunately, not all patients have profited equally; poor education and drug use have been found to be important determinants of treatment effectiveness [22,23], and a recent analysis of EH data showed that regular attendance of HIV clinics, which is likely to be highly correlated with treatment effectiveness, was inversely associated with being homeless, living in institutions, being unemployed, having low educational status and having injected drugs [24]. These results highlight the fact that, even in countries with free access to healthcare and treatment, social determinants play a central role in achieving treatment effectiveness and should be taken into consideration, particularly in a time of economic crisis.

Co-morbidities are a major factor for the quality of life and life expectancy of PLWH. In Spain, due to the central role that injection of drugs had in the initial expansion of the HIV epidemic, hepatitis B and C are the most important co-morbidities, as our results show. Both infections, especially hepatitis B, can also be associated to sexual contact, but this association was not present in our data. Vaccination for hepatitis B is available free of charge in Spain during the entire study period, and the proportion reporting vaccination in our study increased from 17.7% in 2002 to 32.9% in 2011. The prevalence of active infection with HCV in our study (three of 10 PLWH) is similar to that found in other studies in Spain [25]. Although treatment for those infections, including liver transplant, is available, hepatopathies are one of the major causes of mortality in HIV-infected people [26,27].

The information system described in this paper has some limitations. Firstly, patients who attend clinics more regularly and/or those who are more seriously

ill tend to be overrepresented. Secondly, it is possible that patients tend to respond to behavioural questions in a favourable way. Thirdly, questionnaires are administered by many different individuals, making it difficult to control reproducibility and data quality. Fourthly, HIV-infected people who receive care at the participating hospitals for reasons other than HIV are excluded and it is not clear how this could influence the outcomes. Finally, the survey is very sensitive to administrative problems arising on the day of the survey, e.g. a temporary lack of human resources or a strike in the transportation system.

On the other hand, this information system has several strengths. Firstly, it is population-based, allowing all PLWH who attend HIV care in a particular area to participate, rather than those enrolled in cohorts, which often tend to have strict enrolment criteria. Secondly, by providing periodic snapshots across the years it is possible to observe the development of the epidemic over time. Thirdly, clinical staff know the patients and their clinical data very well, allowing them to use existing rapport and clinical knowledge to more easily complete the questionnaire. Last but not least, the entire survey uses existing resources and thus is performed at little to no cost.

Conclusion

In summary, this hospital-based one-day cross-sectional survey of PLWH allows the collection of second generation surveillance data in this group in Spain. This information is useful to inform preventive policies and to plan services, and allows the assessment of treatment effectiveness and the identification of treatment and prevention barriers.

The results of this analysis have identified areas in which HIV prevention and control in Spain can be further strengthened and provide a model for other countries aiming to implement a low-cost and sustainable system for second generation surveillance among PLWH. While clinical results suggest good treatment effectiveness in our setting, efforts should be made to improve condom use among the patients and to further reduce drug injection.

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Conflict of interest

None declared.

Authors' contributions

MD was the main study researcher. She supervised field work and data collection, wrote the statistical analysis plan and the first version of the manuscript. AD did statistical analysis and made important contributions to successive versions of the manuscript. CG performed data collection and management, quality control and reviewed all the manuscript drafts. MP, AT, HM, GG, MCR, JT, GG, RA, AI, LJV, EM, LE, DC, IL were the staff responsible for coordinating the survey in the autonomous regions. They participated in development of the study protocol, supervised field work and estimated the population coverage. They have critically reviewed all versions of the manuscript.

SM, JG-G, AMB, SA, MTG, CR, AC and the Hospital Survey Study Group were the clinicians responsible for patient recruitment the participating hospitals and performed field work in their hospitals. They have reviewed all versions of the paper.

All authors have seen and approved the final manuscript.

*Erratum

The name of A Diaz was left out from the author list on publication. It was added on 23 May 2014. We apologise for this mistake.

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Letter to the Editor: Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels: are dromedary camels a reservoir for MERS-CoV?

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To the Editor:

In a recent issue of *Eurosurveillance*, we read the article by Nowotny et al. [1]. In this article, the authors concluded, based on phylogenetic analysis and a high Middle East respiratory coronavirus (MERS-CoV) load in nasal swabs of dromedary camels, that local zoonotic transmission of MERS-CoV from camels may be possible through the respiratory route. We would like to thank the authors for their contribution to the knowledge of MERS-CoV. However, we would like to report a few concerns regarding this study from a methodological point of view [1].

First, the authors suggested that the high viral load of MERS-CoV detected in nasal swabs may facilitate the zoonotic transmission through the respiratory route. However, in the study, the data on viral loads in dromedary camels were not described in greater detail and were only derived from testing nasal and conjunctival swabs for the virus. Additionally, the use of upper respiratory track swabs instead of lower respiratory specimens may not give a complete picture of the infection, because the MERS-CoV load in upper respiratory tract specimens is reported to be less important than in lower respiratory tract specimens [2].

Second, while it is known that MERS-CoV can cause severe disease and even death in humans, and this infection has no prophylaxis or specific treatment [3], the authors did not give any detailed information about the respiratory route, in particular whether droplet or aerosol transmission may occur. This constitutes a limitation of this study not mentioned by the authors.

Last, contacts and risk of contagion between the 76 dromedary camels, from which the samples were taken, were not provided in detail in the article.

If the mode of transmission is not well known and not understood, clinicians should pay attention to implement the precautionary principles recommended by the Centers for Disease Control and Prevention (CDC) as airborne precautions (the use of respirators rather than surgical masks), in addition to standard and contact precautions to reduce the risks of this infection until clarification of scientific certainty [4].

In previous studies, it was reported that MERS-CoV infection may be transmitted via respiratory droplets or direct and indirect contact with an infected person [5,6,7]. In addition, there has been international concern in the medical community about the risk of MERS-CoV to have a pandemic potential due to aerosol transmission. A recent study demonstrated that there was no evidence of MERS-CoV nasal carriage among Hajj pilgrims [2]. We agree with this study and believe that there is no aerosol transmission of this disease [2]. The result of other work has also shown that MERS-CoV survives in raw camel milk slightly longer than in milk of other species [8].

Among the Middle East countries with a desert climate, camels are still a major means of transportation and trade. The fact that camels may be a reservoir for MERS-CoV [1], and the possibility that camels could spread MERS-CoV infection with pandemic risk to other countries and regions unaffected by this virus, should be taken into consideration. Given the popularity of camel milk consumption and trade in these countries, it would be appropriate to take regulatory measures on import of camels and camel milk from endemic areas, due to the reasons mentioned above.

An issue here should not be misunderstood: previously on the Asian continent, millions of poultry were destroyed due to the pandemic risk of avian influenza A(H5N1) [9]. It is not intended to say that camels, which are claimed to be a reservoir for the disease and play an important role in supplying the basic needs of the people in countries with a desert climate, should be destroyed, but rather it is meant to say that precautionary measures to protect the animals and people should be taken.

In conclusion, MERS-CoV is an emerging pathogen with pandemic potential and with high risk of mortality. It is vital to take all possible preventive measures against MERS-CoV infection. Although in the Nowotny study [1] positive polymerase chain reaction (PCR) results showed MERS-CoV in five of 76 camels, an explicit assessment of the epidemiological role of camels has yet to be made to clarify the mechanism of emergence in humans. Further studies are required to better understand the transmission route and risks of this infection.

Conflict of interest

None declared.

Authors' contributions

Mustafa Hatipoglu investigated and found MERS-CoV data in literature. Ergenekon Karagöz evaluated and criticised these articles in literature. Vedat Turhan supervised all procedures.

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Authors' reply: Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels: are dromedary camels a reservoir for MERS-CoV?

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To the Editor:

We thank Karagöz et al. for their letter [1] in response to our paper [2]. The letter is a good opportunity to provide additional information on Middle East respiratory syndrome coronavirus (MERS-CoV) infection in dromedary camels and their potential role in the transmission of MERS-CoV to humans.

In fact, we mentioned in our paper [2] in detail that the cycle threshold (Ct) values in nasal and conjunctival swabs of the five MERS-CoV reverse transcription-quantitative polymerase chain reaction (RT-qPCR) positive Omani camels ranged from as low as 15.74 to 36.29, indicating a high viral load in the former. Our results were confirmed in a recent report by Raj et al. [3], who demonstrated an even higher viral load (Ct values of 11.3 and 12.9) in a nasal swab of an eight-month-old camel from Qatar, sampled in February 2014. High loads of MERS-CoV nucleic acid in nasal swabs of dromedary camels from Saudi Arabia were also reported by Alagaili et al. [4].

Karagöz et al. [1] pointed out that we investigated specimens from the upper and not the lower respiratory tract, because MERS-CoV load has been reported to be less in upper respiratory tract samples than in lower respiratory tract samples. Although this is indeed true for human patients [5], who frequently develop during the course of a MERS-CoV infection, severe lower respiratory tract disease including pneumonia [6], infection in camels has been reported to be either asymptomatic [7] or associated with only mild respiratory signs with nasal discharge [8,9]. Consequently, the much easier – and nonetheless highly success-oriented – way of sampling is taking nasal swabs, and so far, all studies investigating MERS-CoV in the respiratory tract of camels have been carried out on nasal swabs and not on lower respiratory tract specimens [2-4,7-10]. To further understand many aspects of MERS-CoV infection in camels, including pathogenesis, organ tropism, clinical symptoms, viral loads, and viral shedding, more studies are needed, both in the field and in controlled conditions.

The question of whether droplet or aerosol transmission of the MERS-CoV may occur is currently highly debated [5], mainly for human-to-human but also for camel-to-human transmission. Whether camels excrete and thereby may transmit MERS-CoV only in the form of droplets or also as aerosol can only be addressed in an experimental setting. Delineating the mode of respiratory transmission was not the goal of our study, and it is actually impossible in an epidemiological study to determine this; consequently, it cannot be considered a limitation of the study.

Despite a surge of reported human MERS-CoV infections during April and May 2014, which can be partly attributed to two healthcare-associated clusters and the detection of asymptomatic and mild cases through enhanced surveillance activities, it should be clearly noted that there is currently no risk of a human MERS-CoV pandemic, since the basic reproductive rate of the virus (R_0) is definitely below 1 and probably below 0.5 [11], which excludes sustained human-to-human transmission.

So far there is no report showing that infected camels secrete MERS-CoV in milk. In our opinion, there is no current need to apply regulatory measures on camel milk imports; however, the local population in certain regions should be convinced to abstain from drinking raw camel milk, not necessarily because of MERS-CoV but due to the risk of contracting brucellosis, Q-fever and other known zoonoses transmitted by dromedary camels [12]. We also think that camel meat does not really pose a risk for the consumer if standard hygienic procedures such as washing hands carefully after handling raw camel meat are applied; however, people

slaughtering camels should be advised to wear protective gear, mask and glasses.

MERS-CoV infection in camels is widespread in the Arabian Peninsula, e.g., [13] and Africa [14]; consequently, trade restrictions would not be effective. In order to limit possible MERS-CoV transmission from camels to humans, a MERS-CoV vaccine for camels should be developed and applied to young camels after the levels of maternally derived antibodies decrease. No one has been suggesting culling of camels because of MERS since there is no reason at all for such an approach.

We do apologise that we were unable to report data on age, clinical status, geographical area of sampling, and travel history of the MERS-CoV nucleic acid positive camels, as these data were not provided to us.

Human infections resulting from (probably very close direct) contact with acutely infected camels have been shown [8], and such cases may be the source of limited human-to-human transmissions. However, the vast majority of MERS-CoV transmissions seem to occur within families [15], in the community [16] and in healthcare facilities [15], which especially raise a serious concern. In addition, in a growing number of infected people, the source of infection remains unclear.

We are only at the beginning of our understanding of MERS, and we fully agree that there are still many open questions, including the epidemiological role of camels and the MERS-CoV transmission routes. These unknown areas need to be addressed in joint efforts by the national medical and veterinary authorities of the affected countries, research institutions, and internationally coordinated by the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organisation for Animal Health (OIE).

Conflict of interest

None declared.

Authors' contributions

NN wrote the manuscript; JK read and revised the manuscript.

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Letter to the Editor: Smoking and older age associated with mumps in an outbreak in a group of highly-vaccinated individuals attending a youth club party, the Netherlands, 2012

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To the Editor:

In their recent article, Ladbury et al. present an investigation of an outbreak of mumps in a highly vaccinated group attending a youth club party in March 2012 [1]. The article suggested that crowded social events and smoking may facilitate spread of mumps virus among a highly vaccinated population and that waning immunity may also play a role. We would like to address a number of interesting points.

Firstly, the elevated attack rate (AR) of mumps virus among smokers (41.7%) and non-smokers (15.9%) and the significantly increased risk ratio (RR) (3.1; 95% CI: 1.6–6.0, p=0.001) attributed to smoking at the youth club party is noteworthy in this highly vaccinated cohort when exposure through saliva transmission and sharing of cigarettes was discounted. Exposure to cigarette smoke is known to be associated with a significant increase in the risk of important bacterial and viral respiratory infections. Smokers incur an up to fourfold increased risk of invasive pneumococcal disease and influenza incidence and clinical severity is higher in smokers compared to non-smokers [2]. In vitro, cigarette smoke extracts suppress anti-viral and innate immune responses following infection by respiratory RNA viruses including the respiratory syncytial virus which is a paramyxovirus highly related to mumps virus [3, 4]. Allied with waning immunity to mumps this could potentially contribute to the increased susceptibility of subjects' infection and account for the apparent higher attack rates.

Secondly, the authors reported that another independent risk factor for mumps infection was older age. Respondents aged ≥21 years had a significantly higher AR (54.6%) than those under 21 (14.9%, (RR 3.7; 95% CI: 1.5-8.7, p=0.005). A report from Australia in 2007 identified 76% of mumps notifications as people aged 20 years or older [5], and we have also reported similar findings in two separate mumps outbreaks in highly vaccinated populations in Ireland [6, 7]. Indeed, in the years between outbreaks, mumps cases were highest in persons' ≥30 years of age, suggesting that this may be the cohort maintaining ongoing mumps transmission; however, the mechanisms of continued transmission remain unclear and warrant further study.

Thirdly, the possibility of incomplete humoral protection or waning immunity following mumps vaccination or natural infection suggests that the presence of mumps-specific IgG antibody levels may not prevent re-infection. However, based on the mild clinical symptoms observed by Ladbury et al., one could argue that the response to the vaccine had a clinically protective effect following exposure to the virus. Thus, it is probable also that the viral load in the respiratory tract of these individuals would be significantly lower than that during primary mumps infection, and the risk of onward transmission would be low.

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