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Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene

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During a Brazilian multicentric antimicrobial resistance surveillance study, colistin resistance was investigated in 4,620 Enterobacteriaceae isolated from human, animal, food and environmental samples collected from 2000 to 2016. We present evidence that *mcr-1*-positive *Escherichia coli* has been emerging in South America since at least 2012, supporting a previous report on the possible acquisition of *mcr-1*-harbouring *E. coli* by European travellers visiting Latin American countries.

We present evidence that *mcr-1*-harbouring *Escherichia coli* has been occurring in food-producing animals in Brazil since at least 2012.

Screening Enterobacteriaceae isolates for potential colistin resistance and the *mcr-1* gene

Between 2000 and 2016, a total of 4,620 Enterobacteriaceae isolates were collected in Brazil, as part of different surveillance projects on carbapenemase- and/or extended-spectrum beta-lactamases (ESBL)-producing Gram-negative bacteria important to human and veterinary medicine [1-4]. Within this Brazilian multicentric antimicrobial resistance surveillance study, we hereby also investigate colistin resistance.

The 4,620 isolates were screened using MacConkey agar plates supplemented with colistin (2 mg/L). A total of 515 isolates, which had grown on the screening plates were obtained. These originated from food-producing animals (227 isolates), chicken feed (4 isolates), companion (9 isolates) and non-companion animals (24 isolates), humans (137 isolates), food (102 isolates) and the environment (12 isolates). The 515 isolates were further tested for susceptibility to colistin by agar dilution and/or broth microdilution method, whereby a minimum inhibitory concentration (MIC) >2 mg/L was considered indicative of colistin resistance according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5]. Isolates were also subjected to polymerase chain reaction (PCR) to check whether respective strains harboured the *mcr-1* gene [6], which if present was sequenced (Table).

The *mcr-1* gene was detected in 16 commensal *E. coli* strains exhibiting colistin MICs from 1 to 16 mg/L (MIC_{50} = 8 mg/L). Two of the *mcr-1*-positive *E. coli* strains were found in faecal samples collected in 2012 from healthy pigs in farms located in Santa Catarina and Minas Gerais states. One of these two isolates was susceptible for colistin (MIC = 1mg/L). The remaining 14 *mcr-1*-harbouring *E. coli* strains originated from faecal samples of healthy chickens, which had been gathered in 2013 from farms located in Paraná, São Paulo and Minas Gerais states. All 14 isolates from chickens had a MIC ≥ 8 mg/L.

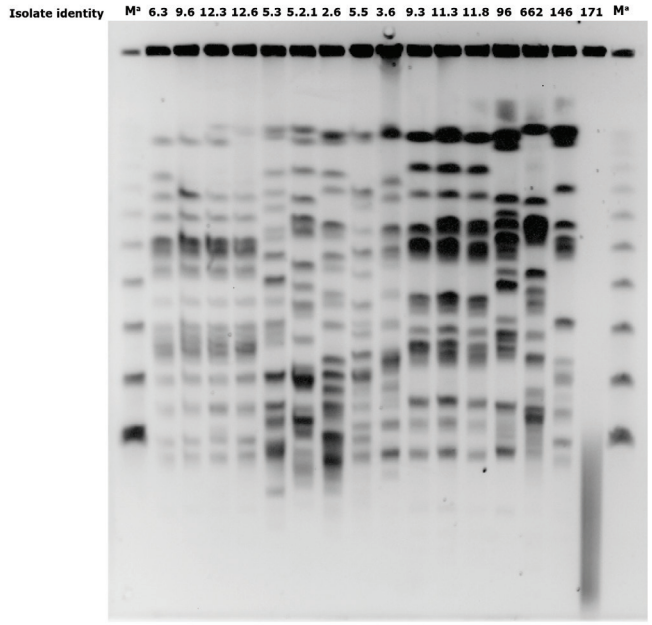
Relationships between *mcr-1*-positive isolates, and testing for extended-spectrum beta-lactamases

The sequences of the 16 *mcr-1*-positive *E. coli* strains were phylogenetically analysed [7], revealing that 11

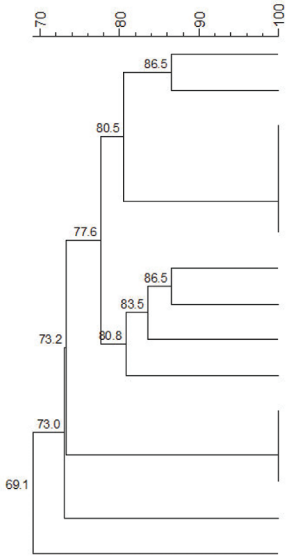
Figure 1

Pulsed-field gel electrophoresis (PFGE) and antimicrobial resistance chraracteristics of *mcr*-1-positive *Escherichia coli* strains isolated from faeces of healthy livestock, Brazil, 2012–2013

A. *XbaI* PFGE of MCR-1-positive *E. coli* strains isolated from faeces of healthy livestock



B. Relationship between isolates obtained after *XbaI* PFGE and antimicrobial resistance



Isolate	Source	Year	State[b]	Resistance profile (Kirby – Bauer)[c]	Colistin MIC (mg/L)[d]	β-lactamase	Phylogroup	PFGE cluster[e]
5.3	Chicken	2013	PR	CRO, CTX, CTF, CPM, TET	8	CTX-M-1	A	A
662	Chicken	2013	SP	–	8	–	A	B
12.3	Chicken	2013	PR	CRO, CTX, TET	16	CTX-M-8	A	C
12.6	Chicken	2013	PR	–	8	–	A	C
6.3	Chicken	2013	PR	CRO, CTX	8	CTX-M-8	A	C
9.6	Chicken	2013	PR	CRO, CTX, CTF, CPM, ENO	8	CTX-M-8	A	C
2.6	Chicken	2013	PR	CRO, CTX, TET	8	CTX-M-15	A	D
3.6	Chicken	2013	PR	TET	8	–	B1	E
5.5	Chicken	2013	PR	CRO, CTX, CTF, CPM, TET	8	CTX-M-15	A	F
146	Swine	2012	SC	AMC, FOX, CLO, SXT, TET	1	CMY-2	A	G
11.3	Chicken	2013	PR	CRO, CTX, CTF, CPM, CIP, ENO	8	CTX-M-8	B1	H
11.8	Chicken	2013	PR	CRO, CTX, CTF, CPM, CIP, ENO	8	CTX-M-8	B1	H
9.3	Chicken	2013	PR	CRO, CTX, CPM, CIP, ENO	8	CTX-M-8	B1	H
96	Chicken	2013	MG	ENO, CIP	8	–	A	I
5.2.1	Chicken	2013	PR	ENO	8	–	A	J
171	Swine	2012	MG	CLO, SXT	8	–	B1	nt

MIC: minimum inhibitory concentration; nt: non typeable by PFGE.

GenBank accession number for mcr-1 genes identified in this study: KU750813, KU928239–42, KU935441–9, KX01152–1.

a The marker (M) used was the Lambda ladder 0.05–1Mb, Bio-Rad. Separation of fragments was carried out at 6V/cm at 14°C for 20h, with linear pulse time of 3.51s to 30.82s.

b The states were as follow: MG: Minas Gerais state (South-east Brazil); PR: Paraná state (South); SC: Santa Catarina state (South); SP: São Paulo (South-east).

c The antimicrobial susceptibility was evaluated by disc diffusion assay. Extended-spectrum beta-lactamase (ESBL) production was investigated by using a double-disc synergy test (DDST) [5,23,24]. AMC: amoxicillin/clavulanic acid; CAZ: ceftazidime; CFX: cefoxitin; CIP: ciprofloxacin; CLO: chloramphenicol; CPM: cefepime; CRO: ceftriaxone; CTF: ceftiofur; CTX: cefotaxime; ENO: enrofloxacin; FOS: fosfomycin; GEN: gentamicin; SXT: trimethoprim/sulfamethoxazole; TET: tetracycline.

d MICs were determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5,25]. Colistin resistance was defined as a colistin MIC› 2 mg/L, according to EUCAST clinical breakpoints [5].

e PFGE patterns were analysed using the Dice similarity with coefficient optimisation set at 1% and tolerance at 2% (BioNumerics software; Applied Maths, Kortrijk, Belgium).

Figure 2

Geographical distribution of *mcr*-1-positive *Escherichia coli* isolates reported from South America, 2012–2016

A light grey colour is used for Brazil, where this study was conducted. The dark grey colour indicates countries (Bolivia, Colombia and Peru) visited between November 2012 and November 2013, by unrelated Dutch travellers, for whom acquisition of faecal colonisation and carriage with MCR-1 and extended-spectrum beta-lactamase (ESBL)-producing E. coli was shown one to two weeks after their return to the Netherlands [12]. A dark grey colour is used for Ecuador, where subsequent to the identification of a human mcr-1-positive isolate, a sequence was deposited in GenBank in March 2016 (GenBank accession number: KU886144.1).

Table

Results of screening *Enterobacteriaceae* isolates from different sources by culture with colistin and presence of the *mcr*-1 gene in the screened isolates, Brazil, 2000–2016 (n = 4,620 isolates screened)

Source[a]		Years of isolate collection	Enterobacteriaceae isolates tested *n*	Enterobacteriaceae isolates with growth on screening plates (2 mg/L colistin) *n*[b]	Isolates positive for *mcr-1* *N (% of isolates screened)*[c]
Food-producing animals	Chicken	2003–2015	280	113	14 (5.0)
	Swine	2012–2014	113	79	2 (1.8)
	Cattle	2014–2015	158	22	0 (0)
	Goat	2013	7	1	0 (0)
	Ostriches	2015	9	2	0 (0)
	Buffalo	2010	36	10	0 (0)
Chicken feed	–	2000–2014	8	4	0 (0)
Companion animals	Cats	2013	4	0	0 (0)
	Dogs	2013	51	9	0 (0)
Non-Companion animals	Horse	2013	13	3	0 (0)
	Rodents	2013–2014	14	13	0 (0)
	Turtle	2015	21	8	0 (0)
	Urban pigeons	2015–2016	36	0	0 (0)
	Urban waterfowl	2012–2014	75	0	0 (0)
Human infection/ colonisation	–	2004–2016	3,591	137	0 (0)
Food	Chicken meat	2013	42	22	0 (0)
	Swine meat	2012–2014	113	79	0 (0)
	Cabbage	2016	2	0	0 (0)
	Lettuce	2016	2	0	0 (0)
	Spinach	2016	1	1	0 (0)
Environment	Lake	2012–2013	20	2	0 (0)
	River	2011	3	3	0 (0)
	Sewage	2009–2013	21	7	0 (0)
Total	**–**	**–**	**4,620**	**515**	**16 (0.3)**

[a] Isolates originated from previous surveillance studies of carbapenemase- and/or extended-spectrum beta-lactamases (ESBL)-producing Gram-negative bacteria in food, food-producing animals (faecal samples from healthy animals), chicken feed, companion and non-companion animals (faecal samples from healthy animals), environment and human patients from healthcare settings (27 faecal samples from colonised individuals and 3,564 clinical cultures from infections), all collected in Brazil between 2000 and 2016 [1-4].

[b] Isolates were screened for potential colistin resistance using MacConkey agar plates supplemented with colistin (2 mg/L).

[c] Enterobacteriaceae isolates with growth on screening plates were subjected to *mcr-1* polymerase chain reaction and sequencing [6].

strains belonged to the phylogroup A and five to the phylogroup B1. Clonal relatedness of the strains were further determined by *XbaI* pulsed-field gel electrophoresis (PFGE) (www.cdc.gov/pulsenet/). PFGE differentiated *mcr-1*-positive *E. coli* isolates into 10 distinct pulsotypes (named A to J), which clustered into two major groups, C (n = 4) and H (n = 3) (Figure 1).

The 16 *mcr-1*-positive isolates were additionally tested for the production of extended-spectrum beta-lactamases (ESBLs) by using a double-disc synergy test (DDST) as well as for the presence of ESBL- and plasmid-mediated AmpC (pAmpC) beta-lactamase genes [1,6].

Most (n= 9) *mcr-1*-positive isolates exhibited resistance to human and/or veterinary cephalosporins. In this regard, such isolates harboured $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-8}}$ and/or $bla_{\text{CTX-M-15}}$ ESBL genes, and one isolate carried the pAmpC $bla_{\text{CMY-2}}$ gene. On the other hand, all isolates carrying the *mcr-1* gene belonged to low-virulence *E. coli* phylogroups (i.e. A and B1 as described above).

Discussion

The plasmid-mediated colistin (polymyxin E) resistance mechanism MCR-1 was first described in Enterobacteriaceae isolated from animals, food and human beings in China [6]. Since, and as summarised by Skov and Monnet [8], MCR-1 has also been reported to occur in other countries in Asia, Europe and North America. Recent descriptions from Egypt [9], Italy [10]

and Spain [11] further denote dissemination of the mechanism, while identifications of *mcr-1* positive strains in imported food, urban rivers and travellers [12-16] highlight the potential for MCR-1 to continue spreading. In addition, co-production of ESBLs or carbapenemases by *mcr-1*-harbouring Enterobacteriaceae has now been documented [12,13,15-18].

We report *mcr-1*-positive *E. coli* isolates from food-producing animals in the southern (Santa Catarina and Paraná states) and south-eastern (São Paulo and Minas Gerais states) regions of Brazil (Figure 2). Interestingly, in most of these isolates (9 of 16), *E. coli* strains co-produced CTX-M-type ESBLs.

Our findings moreover suggest that *mcr-1*-harbouring *E. coli* strains have been present in South America since at least 2012, supporting the results of a previous study on the possible acquisition of *mcr-1*-carrying *E. coli* by European travellers visiting this continent (Figure 2) [12]. In this previous prospective study, the carriage of multiresistant bacteria after travel (COMBAT) consortium had shown that unrelated Dutch travellers to Bolivia, Colombia and Peru between November 2012 and November 2013 had become carriers of/colonised with MCR-1 and ESBL-producing *E. coli* one to two weeks after their return to the Netherlands [12].

Recently the *mcr-1* gene has also been identified in another Latin American country, Ecuador, whereby a respective sequence from a human clinical *E. coli* isolate was submitted to GenBank (GenBank accession number: KU886144.1) in March 2016. Therefore, hospital laboratories worldwide should be aware of the possibility of MCR-1 in Enterobacteriaceae isolates resistant to polymyxins from patients living in or returning from Latin American countries.

That *E. coli* with plasmid-mediated MCR-1 are found in Brazil is also relevant for medical centres in this country, where the emergence and dissemination of multidrug-resistant pathogens, which is associated with high rates of treatment failure, have led to high use of polymyxins, mainly in intensive care units [19]. There, this class of antimicrobial agents represents the main therapeutic option for treating severe 'superbug' infections, particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* producing SPM-1, OXA-23 or KPC-2 carbapenemases, which are highly prevalent in most Brazilian hospitals [19]. On a positive note however, our study did not find *mcr-1*-positivity in any of the human isolates screened, which is consistent with the very low background carriage of MCR-1 in humans, as described previously [6,12-14].

Our result that the *mcr-1* gene occurs in Brazilian livestock is a cause for concern in terms of the global contribution of Brazil to national and international movement of people and products, as this could contribute to the acceleration of the worldwide spread of the *mcr-1* gene. Indeed, with a population of 205 million inhabitants, Brazil has continental proportions and is the biggest country in Latin America. Furthermore, in the agribusiness it is the third producer of chicken meat (only after the United States and China) and the largest exporter of this product [20]. In this regard, colistin sulphate is widely used in animal feed as a growth promoter in Brazilian livestock, mainly in pigs and poultry, supporting a link between the agricultural use of colistin and colistin resistance [21].

Finally, the identification of a colistin-susceptible *E. coli* strain carrying the *mcr-1* gene, in this study, suggests that *mcr-1*-positive isolates may be difficult to detect if the *mcr-1* gene is only tested for in colistin resistant isolates. This may contribute to the silent dissemination of *mcr-1* harbouring strains. In fact, many MCR-1 producers are known to exhibit low level of resistance to colistin (i.e. 4–16 mg/L) [6,8-14,16,22].

In summary, since MCR-1-producing strains have already become established in South America, we emphasise the need for continuous local surveillance programmes to identify the risk to human health. To reduce this risk, the authors suggest that colistin should only be used for treatment of clinical infectious diseases and no longer for animal production, in order to prevent the wide spread of MCR-1-producing bacteria, achieving the principles of responsible use of antibiotics.

Erratum

The term '*mcr-1*' had been mistyped as '*mrc-1*' on several occasions and this was corrected on 02 May 2016.

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Conflict of interest

None declared.

Authors' contributions

MRF, QM, LS, FE, RL, LKO, DDG, MD, MHM, DFMM, ML, DdOG, TK and AMM collected the data and samples, MRF, QM, LS, KCS, MPVC, FE, RL, MD, GRF, MFCB and NL performed the microbiological and molecular analysis, MRF, QM, KCS, FE, MD, DdOG, TK and NL participated in drafting the manuscript, NL coordinated and edited the manuscript.

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Ongoing outbreak of invasive listeriosis due to serotype 1/2a *Listeria monocytogenes*, Ancona province, Italy, January 2015 to February 2016

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In the first seven weeks of 2016, five serotype 1/2a *Listeria monocytogenes* isolates were collected from patients with invasive listeriosis in Ancona province in Italy. These strains and six 1/2a isolates identified in 2015 in the same area were typed by ERIC-PCR and PFGE. A clonal relationship, documented between the two sets of isolates, suggested a listeriosis outbreak in Ancona that started most probably in 2015. Investigation into the source of infection is still ongoing.

In the first seven weeks of 2016, six cases of invasive listeriosis were recorded in Ancona province, Italy. Five strains of *Listeria monocytogenes* serotype 1/2a were isolated and typed by enterobacterial repetitive intergenic consensus (ERIC)-PCR and PFGE, indicating clonality. In addition, seven serotype 1/2a *L. monocytogenes* strains from cases of invasive listeriosis recorded in the same area in 2015 were also typed and showed relatedness. Here we provide details of the ongoing outbreak.

Outbreak description

From 4 January to 15 February 2016, six *L. monocytogenes* strains (3 from blood, 3 from cerebrospinal fluid (CSF)) were isolated from six patients diagnosed with invasive listeriosis at the Clinical Microbiology Laboratory of Ancona Regional Hospital (eastern Italy) of Area Vasta 2 (AV2) which encompasses Ancona, Fabriano, Senigallia, and Jesi. Patients had been admitted to four different departments: emergency room (ER) (n=2), oncology (n=2), infectious diseases (n=1), and intensive care unit (ICU) (n=1). Four of the six patients were women and the most common risk factors/underlying conditions were: age (n=5; >71 years), cancer (n=2), and diabetes (n=1). Clinical manifestations included septicaemia (n=3), meningitis (n=2) and meningoencephalitis (n=1).

In addition to the cases detected in 2016, eight *L. monocytogenes* strains (5 from blood and 3 from CSF) had been isolated in AV2 (from 7 cases) and nearby Ascoli Piceno (from 1 case) in 2015 (Figure 1); clinical samples came from six hospital departments: ER (n=1), general medicine (n=3), nephrology (n=1), vascular surgery (n=1), infectious diseases (n=1), and ICU (n=1). Five patients were men and the mean patient age was 73.6 years (range: 55–84; median: 75); a 77 year-old man died.

The 2015 and 2016 isolates were identified as *L. monocytogenes* by Gram staining and the Vitek MS system (bioMérieux Italia SpA, Firenze, Italy). Susceptibility to ampicillin, meropenem, erythromycin, and sulphamethoxazole-trimethoprim was tested by the E-Test (Liofilchem, Teramo, Italy) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [1]. All strains were susceptible to all the antibiotics tested.

Molecular typing

In order to identify relatedness, the 2015 and 2016 *L. monocytogenes* isolates were sent to our laboratory (Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona) for molecular typing. Multiplex PCR serotyping [2] assigned five 2016 isolates and seven 2015 isolates to serotype 1/2a; the remaining isolates were serotype 4b (2016) and serotype 1/2c (2015).

Genetic relatedness was explored by ERIC-PCR [3] and by PFGE after ApaI digestion of total DNA [4]. ERIC pattern similarity was determined on the basis of the Dice similarity coefficient; the matrix thus generated was subjected to clustering using TREECON software (Bioinformatics and Evolutionary Genomics, Gent, Belgium). The 1/2a 2015 and 2016 isolates shared a

Figure 1

Time distribution of listeriosis cases, Ancona and Ascoli Piceno, January 2015 to February 2016 (n=14)

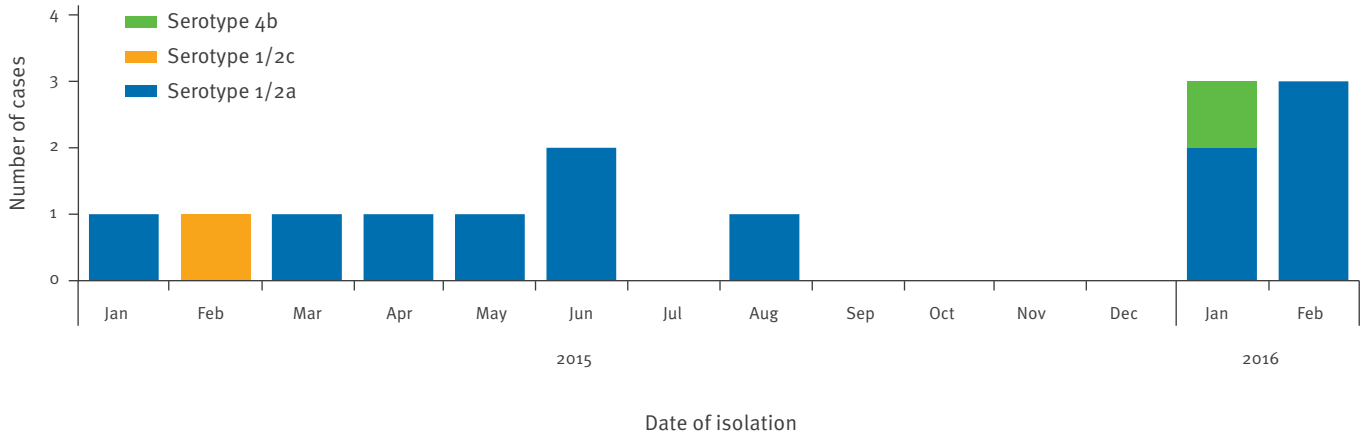
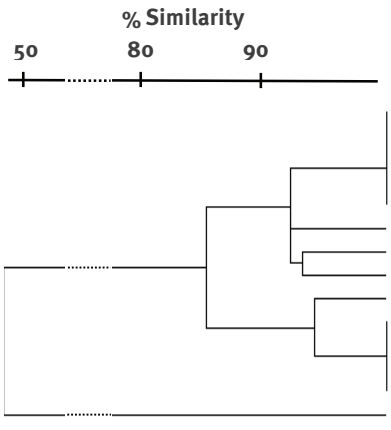


Figure 2

Enterobacterial repetitive intergenic consensus (ERIC)-PCR-based dendrogram showing the similarity index among the *Listeria monocytogenes* isolates, Ancona province, January 2015 to February 2016 (n=14)



Strain	Date of isolation	Age of patient	Source	Serogroup (serotype)
14435	09 Mar 2015	75	Blood	IIa (1/2a)
27543	12 May 2015	75	CSF	IIa (1/2a)
48923	31 Aug 2015	77	CSF	IIa (1/2a)
31829	02 Jun 2015	73	Blood	IIa (1/2a)
32725	09 Jun 2015	55	CSF	IIa (1/2a)
02470	03 Jan 2015	84	Blood	IIa (1/2a)
09707	12 Feb 2015	76	Blood	IIc (1/2c)
56053	08 Apr 2015	Unknown	Blood	IIa (1/2a)
73844	04 Jan 2016	83	Blood	IIa (1/2a)
76622	19 Jan 2016	72	Blood	IIa (1/2a)
80466	08 Feb 2016	56	Blood	IIa (1/2a)
80864	10 Feb 2016	83	CSF	IIa (1/2a)
81753	15 Feb 2016	83	CSF	IIa (1/2a)
77660	25 Jan 2016	78	CSF	IVb (4b)

CSF: cerebrospinal fluid.

The scale at the top indicates the degree of genetic relatedness.

five-band pattern ranging from 1,500 to 900 bp (Figure 2).

Moreover, four of five serotype 1/2a 2016 isolates (76622, 80466, 80864, 81753) displayed identical ERIC PCR profiles; the remaining isolate (73844) differed by one band (›90% similarity index). The profile of the serotype 4b strain (77660) was completely different (‹50% similarity index). All 1/2a 2015 isolates showed a high degree of similarity (›85%) with respect to the 1/2a 2016 isolates. Notably, the profile of the single serotype 1/2c isolate (09707) was closely related to that of the 1/2a isolates.

PFGE analysis confirmed ERIC PCR results, except for two 1/2a isolates, i.e. strain 56053 (Ascoli Piceno) and strain 02470 (the first 2015 isolate) (data not shown). The DNA of serotype 1/2c strain 09707 was not digested by ApaI.

Background

L. monocytogenes is widely distributed in the environment and is frequently isolated from a variety of sources, including soil, vegetation, food of animal origin such as meat and dairy products, silage, fecal material, sewage, and water [5]. Listeriosis is most often transmitted through food and primarily affects older adults, pregnant women, newborns, and adults with weakened immune systems [5]. Serotyping is a universally accepted typing method for *L. monocytogenes*, with more than 14 serotypes being recognised according to variation in somatic (O) and flagellar (H) antigens [6]. Multiplex PCR serotyping is a practical alternative to slide agglutination serotyping, since it differentiates among the five major serogroups, each of which

includes multiple serotypes: serogroup IVb (serotypes 4b, 4d and 4e), serogroup IIa (serotypes 1/2a and 3a), serogroup IIb (serotypes 1/2b, 3b and 7), serogroup IIc (serotypes 1/2c and 3c), and serogroup IVa (serotypes 4a and 4c). By use of suitably designed primer pairs, the four major serotypes 1/2a, 1/2b, 1/2c, and 4b produce four distinct PCR profiles [2]. PFGE is considered as the gold standard molecular typing approach for *L. monocytogenes*, owing to its high reproducibility and discrimination ability [4]. ERIC PCR is a relatively simple, cost-effective, and discriminatory typing method based on ERIC sequences, 124 to 127 base-long elements consisting of highly conserved central inverted repeats found in the extragenic regions of the bacterial genome [3].

Discussion and conclusion

The incidence of listeriosis has been rising since the early 2000s in several European countries, mainly in immunocompromised patients older than 65 years [7-9]. In particular, a statistically significant increase was reported in Austria, Denmark, Hungary, Italy, France, Spain, and Sweden from 2005 to 2009 [10]. In the past 30 years, outbreaks of listeriosis have been mostly linked to serotype 1/2a and 4b clones [8]. A shift to serotype 1/2a has been observed in Europe and North America in the last decade [8]. In Italy, surveillance of invasive listeriosis has found an increase in serotype 1/2a isolates over the same period, mainly in the central and northern regions (about 80% of cases) [10-14].

Listeriosis is an infection of great concern to public health due its clinical severity and high case fatality rate, despite its low incidence compared with other foodborne diseases such as salmonellosis or campylobacteriosis. The present data suggest an ongoing outbreak of listeriosis due to serotype 1/2a *L. monocytogenes* in AV2 that most probably started in 2015, since the strain was already present in the area in 2015. As in other European countries, most cases were associated with an underlying condition and involved elderly people [8,9]. Local authorities are working with the Italian national public health institute (the Istituto Superiore di Sanità, Rome) and the regional Istituto Zooprofilattico Umbria and Marche to identify the sources of food contamination. A recent press release [15] points out that there are findings which suggest contamination of a pork product as a possible vehicle of infection for at least one human case. At present, however, no clear link can be established between the contaminated pork product and the infections. Investigation into the source of infection in AV2 is still in progress.

Conflict of interest

None declared.

Authors' contributions

E. Marini, G. Magi and B. Facinelli designed and developed the experimental design. E. Manso and C. Vincenzi collected bacterial strains and epidemiological data; E. Marini and G. Magi performed experiments; B. Facinelli, E. Marini and G. Magi performed data analysis and wrote the manuscript. All authors reviewed and approved the study.

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Contamination during production of heater-cooler units by *Mycobacterium chimaera* potential cause for invasive cardiovascular infections: results of an outbreak investigation in Germany, April 2015 to February 2016

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Invasive infections with *Mycobacterium chimaera* were reported in patients with previous open chest surgery and exposure to contaminated heater-cooler units (HCUs). We present results of the surveillance of clinical cases and of contaminated HCUs as well as environmental investigations in Germany up until February 2016. Clinical infections occurred in five male German cases over 50 years of age (range 53–80). Cases had been exposed to HCUs from one single manufacturer during open chest surgery up to five years prior to onset of symptoms. During environmental investigations, *M. chimaera* was detected in samples from used HCUs from three different countries and samples from new HCUs as well as in the environment at the manufacturing site of one manufacturer in Germany. Our investigation suggests that at least some of the *M. chimaera* infections may have been caused by contamination of HCUs at manufacturing site. We recommend that until sustainable measures for safe use of HCUs in operation theatres are implemented, users continue to adhere to instructions for use of HCUs and Field Safety Notices issued by the manufacturer, implement local monitoring for bacterial contamination and continuously check the websites of national and European authorities for current recommendations for the safe operation of HCUs.

Introduction

In July 2014, the Federal Office of Public Health Switzerland (FOPH) reported about patients with *Mycobacterium chimaera* infections, who had previously undergone open-chest heart surgery with exposure to contaminated heater-cooler units (HCUs) [1]. Several other reports and publications have suggested since that HCUs produced by one manufacturer in Germany may be a source of *M. chimaera* infections that occurred in Switzerland, Germany, the Netherlands and United Kingdom [2-5].

HCUs are commonly used in cardiac surgery during extracorporeal circulation in order to regulate the temperature of the blood and to provide temperature-controlled water for cardioplegia. HCUs have water tanks that provide temperature-controlled water to external heat exchangers. Since *M. chimaera* was detected in air samples close to operating HCUs, airborne transmission is believed to be the most likely transmission mechanism in the *M. chimaera* cases after open chest surgery [4,6].

M. chimaera is a slow-growing nontuberculous mycobacterium (NTM) belonging to the *M. avium* complex (MAC). It was first reported by Tortoli et al. in 2004 as a closely to *M. intracellulare*-related distinct species [7]. Identification requires molecular diagnostic testing [8]. *M. chimaera* may cause lung infections especially in patients with underlying lung disease as well as disseminated infections in immunocompromised patients and was found in skin and bone infections. In the environment, it was identified in biofilms and detected in water sources such as household water [9].

Among others, the report by the FOPH about the outbreak investigations in Switzerland and the reports about cases in Germany and the Netherlands led to increased surveillance efforts and outbreak investigations in Europe [3,10]. Here we present the results of the surveillance of clinical cases, of the surveillance of contaminated HCUs and of environmental investigations in Germany.

Table 1

Cases with symptomatic *Mycobacterium chimaera* infection, notified between April 2015 and February 2016, Germany (n=5)

Case number	Age (years)	Sex	Cardiac surgery centre	Type of surgery (exposure)	Prosthetic material	Site of infection	Death due to infection	Incubation period (years)[a]
1	80	Male	A	Aortic valve replacement	Yes	Endocarditis	No	<1
2	75	Male	B	CABG	No	Spondylodiscitis	No	5
3	65	Male	C	Aortic valve replacement	Yes	Valvular aortic endocarditis, paravalvular leak and abscess	Yes	3
4	67	Male	C	CABG and aortic valve replacement	Yes	Paravalvular abscess[c]	No[b]	4
5	53	Male	C	Aortic valve replacement	Yes	Endocarditis and cerebral abscesses	No	3

CABG: coronary artery bypass grafting.

[a] Time between exposure to open chest surgery involving use of an HCU and clinical diagnosis.

[b] Currently in palliative care.

[c] Endocarditis lenta and change of aortic valve in September 2013.

Methods

Definitions

For our investigations we used the following case definitions: a confirmed case was defined as a patient having undergone surgery with extracorporeal circulation in the five years before onset of symptoms of NTM infection AND in whom *M. chimaera* was detected in an invasive sample (e.g. blood, tissue biopsy or implanted prosthetic material). A probable case was defined as a confirmed case, but without detection of *M. chimaera* in an invasive sample.

An HCU was considered as contaminated, when cardiac surgery centres found NTM and/or other bacteria from environmental samples from the HCU and sent a report to the Federal Institute for Drugs and Medical Devices (BfArM) in Germany.

Prospective case finding and identification of contaminated HCUs

Prospective case finding was conducted from April 2015 onwards and results until end February 2016 are presented here. The mandatory surveillance of healthcare-associated outbreaks in Germany was applied for reporting clinical cases and this surveillance is described in detail elsewhere [11].

The public health authorities and healthcare professionals in Germany were informed about the ongoing outbreak and requested to notify cases fulfilling the case definition [12]. Specifically, the German National public health institute (Robert Koch Institute (RKI)), the German Society of Thoracic and Cardiovascular Surgery and the German Society of Infection informed federal states' authorities and societies' members, respectively, about case definitions and notification according to the article 6 of the 'Protection against Infection Act' (Infektionsschutzgesetz, IfSG) [12-15].

The mandatory notification system for incident reports of medical devices was used to detect contaminated HCUs in Germany. Incident reports were collected and analysed by BfArM in accordance with the corresponding legal framework 'The Act on Medical Devices' (Medizinproduktegesetz) and 'The Medical Device Safety Plan' (Medizinprodukte Sicherheitsplanverordnung).

HCU users were requested to submit any incident report associated with HCUs to BfArM [16]. On 10 July 2015, the BfArM recommended to place HCUs outside of the operation theatre and monitoring of contamination in HCUs [17].

At the European level, the European Centre for Disease Prevention and Control (ECDC) assessed the risk of invasive cardiovascular infection by *M. chimaera* potentially associated with heater-cooler units used during cardiac surgery in Europe also, in April 2015 [10]. The risk assessment was forwarded to regional German public health authorities. From April 2015 onwards, ECDC also provided a platform for exchange of information and a protocol for case detection and environmental testing [18]. The protocol was shared with all European Union/European Economic Area (EU/EEA) countries with the purpose to obtain information in a harmonised way, to further investigate the association between invasive infection by *M. chimaera* and HCUs, and to allow assessing the burden of these infections. The protocol was shared with the German heart surgery centres that detected clinical cases.

Table 2

Mycobacterium chimaera-positive samples from environmental investigations at the manufacturing site of new HCUs and of used HCUs from at the manufacturer's service centre, July 2014 to June 2015

Date	Type of sample	Source of sample
16 Jul 2014	Water (100 mL)	Used HCU from Switzerland
29 Jul 2014	Water (100 mL)	New HCU from manufacturing site
5 Aug 2014	Water (100 mL)	New HCU from manufacturing site
11 Aug 2014	Water (100 mL)	New HCU from manufacturing site
19 Feb 2015	Water (100 mL)	Used HCU from the Netherlands
10 Jun 2015	Water (volume not specified)	Sample taken in pump assembly area at the manufacturing site

HCU: heater-cooler unit.
The environmental investigations were performed by the manufacturer.

Investigation at the HCU manufacturing site and at the manufacturers' service centre

In July 2015, the Bavarian Health and Food Safety Authority (LGL), assisted the Bavarian regulatory authorities with on-site investigations and took environmental samples at the manufacturing site and in the service centre of the implicated manufacturer. Samples were taken from the production line, on-site tap water and from a used and disassembled HCU from this manufacturer in the service centre. All samples were sent to the National Reference Centre (NRC) for Mycobacteria Borstel, Germany.

On its own initiative, the HCU manufacturer conducted environmental sampling for NTM at the manufacturing site where the HCUs are assembled and in the service centre where used HCUs are disassembled for decontamination from July 2014 onwards. Environmental samples were sent to a local microbiological laboratory and NTM isolates were submitted to the NRC in Borstel for further analysis.

Culturing and typing

Mycobacteria were cultured in different laboratories. The development of standard protocols for microbiological *M. chimaera* diagnostic was coordinated by ECDC in collaboration with laboratories such as the NRC Borstel in Europe; these protocols were published by ECDC in August 2015 [18].

Next generation sequencing (NGS) of isolates is still ongoing.

Ethics

A formal ethical review process and approval was not required for this outbreak investigation in accordance with article 25, section 1 of the IfSG.

Results

At the beginning of our investigation, in April 2015, we were informed by cardiac surgery centre A in Germany about a confirmed case that became symptomatic before 2015 [3]. During April 2015 to February 2016, the mandatory surveillance of healthcare-associated outbreaks identified four additional confirmed cases of *M. chimaera* infection who had been exposed to an HCU in two different cardiac surgery centres (B and C) in Germany (Table 1). These cases developed a symptomatic *M. chimaera* infection five months to five years after exposure to a HCU. All five confirmed cases were male and aged above 50 years (range 53–80) when diagnosed with *M. chimaera* infection, four had aortic valve replacement and two underwent coronary artery bypass grafting, one died. All had been exposed to HCUs from one single manufacturer during open chest surgery. No cases with NTM infections other than *M. chimaera* were notified. Our investigations did not reveal epidemiological links between cases of the different sites.

Between January 2015 and February 2016, the BfArM received 26 incident reports of contaminated HCUs from 16 of the total of 78 German cardiac surgery centres from different German regions. Three of the 16 centres reported contamination of HCUs of another manufacturer but *M. chimaera* detection from these HCUs was not reported. Overall, the contaminations of the HCUs included *M. chimaera* and other bacteria such as *Pseudomonas aeruginosa, Legionella pneumophila* and *Stenotrophomonas maltophilia* and fungi. All three centres in which German cases were exposed sent incident reports about contamination of HCUs from the same German manufacturer. Two of these centres reported *M. chimaera* detection in HCU water samples including one reporting also detection of *M. chimaera* in air samples. The third centre reported NTM in HCU water samples, results of further specification were not reported.

During the environmental investigations performed by the Bavarian regulatory authorities on 2 July 2015, six of 20 samples obtained were *M. chimaera*-positive. All positive samples were from one disassembled HCU that had been used in cardiac surgery centre D in Germany and was disassembled for decontamination in the service centre of the manufacturer. The disassembled HCU was produced before modifications in the post-production process that were implemented

by the manufacturer in response to the findings of *M. chimaera* contamination. The samples included in the investigations were water (ca 100 mL), swab and biofilm and were collected from different sources: residual water, filler neck, patient bridge, biofilm from patient recirculation and patient bath.

In December 2015, the HCU manufacturer provided the RKI with information about six *M. chimaera*-positive samples from environmental investigations conducted between July 2014 and June 2015, including two contaminated HCUs from Switzerland and the Netherlands, respectively (Table 2).

On 22 December 2015, public health authorities in the EU/EEA and worldwide were notified by Germany about the suspected common source of *M. chimaera* via the EU Early Warning and Response System (EWRS) and via an International Health Regulation (IHR) notification.

Discussion

We present data that show that *M. chimaera* was isolated in clinical samples from (i) infected patients in Germany who had undergone open chest surgery, (ii) in samples from used HCUs from three different countries and (iii) in samples from new HCUs and the environment at the manufacturing site of one manufacturer. This suggests that at least some of the five German cases with *M. chimaera* infection may have occurred due to contamination of the HCUs by *M. chimaera* at the manufacturing site.

Preliminary typing results indicate that the *M. chimaera* isolates detected by the authorities and the isolates from the manufacturer appear to be almost identical (unpublished data). The *M. chimaera*-positive environmental samples at the manufacturing site prompted the manufacturer to modify the manufacturing process, which now includes ethanol disinfection and an active drying of the HCU water circuit before shipment. When the Bavarian regulatory authorities conducted onsite visits, no *M. chimaera*-positive sample was recovered except from a used HCU which had been disassembled for decontamination. The returned unit had been manufactured before August 2014. According to the information provided by the manufacturer, HCUs manufactured before mid-August 2014 may have had environmental mycobacteria presence in the unit at the time of delivery. Our investigations could not elucidate if and until when contaminated HCUs may have been delivered to customers from this manufacturer.

As of end of March 2016, two additional notifications of patients with *M. chimaera*-positive clinical specimens are under investigation in Germany. Until now we could not obtain data on all surgical interventions prior the *M. chimaera* diagnosis of these patients.

A limitation of our study is that we did not conduct active case finding. It is likely that the passive surveillance has led to an underestimation of the actual number of cases of *M. chimaera* infections in Germany. Furthermore, the true number of cases is probably underestimated since there is no typical clinical picture for infections with *M. chimaera*. Patients present with nonspecific symptoms, a variety of infection sites and a culture for mycobacteria is usually not part of a routine diagnostic work-up in patients presenting with signs of infection.

M. chimaera was not the only bacterial species isolated from HCUs. Contamination of HCUs with other bacteria was reported from various cardiac surgery centres in Germany. Furthermore, bacteria were also isolated from HCUs produced by other manufacturers. It is possible that some of the cases were infected due to contamination of HCUs at the cardiac surgery centres. It is also possible that some of the cases occurred due to exposure to HCUs produced by other manufacturers.

Infections by *M. chimaera* are rare and their occurrence, when detected, is considered unusual [19]. The reported *M. chimaera* infections might therefore be regarded as an indicator of a potential microbial hazard caused by the water-bearing HCUs in the healthcare environment.

Further investigations are needed to differentiate between the risk of *M. chimaera* infection from HCUs contaminated at the manufacturing site, the risk of infection from HCUs contaminated during use and the risk of infection from other medical devices that include an HCU such as extracorporeal membrane oxygenators [20]. In two recent publications, Götting et al. and Sommerstein et al. gave interesting insights into possible mechanisms of airborne transmission by HCUs [4,6]. In the cases described here, NGS should help determine the fraction that may be due to contamination at the manufacturing site or during use at the cardiac surgery centres.

To allow for targeted public health action, it is important that manufacturers of medical products share the findings of their own investigations into bacterial contamination, as demonstrated in this outbreak investigation. Sharing the results by the manufacturer, as well as information on the implemented corrective measures, allowed us to better understand the risks involved in HCU use. Regulatory authorities in Germany are continuing their information exchange with the manufacturers that produce HCUs to provide a sustainable solution for minimising the risks of infection in patients exposed to HCUs.

Conclusions

We present evidence on *M. chimaera* detection in clinical samples from infected German patients having been exposed to HCUs produced by the same manufacturer, in three cardiac surgery centres, in samples from used HCUs from three different countries and in samples from new HCUs and the environment at the manufacturing site of one manufacturer. In summary,

this suggests a point source for the reported *M. chimaera* infections and for *M. chimaera*-positive samples from HCUs and the environment. Notifications of contaminated HCUs of different manufacturers and with various bacteria, indicate a general problem with water-bearing systems in the healthcare environment.

We recommend that until sustainable measures for a safe use of HCUs in operation theatres are implemented, users continue to adhere to the instructions for use of the HCU and the Field Safety Notices issued by the manufacturer, implement a local monitoring for bacterial contamination of the HCUs and continuously check the websites of relevant national and European authorities for current recommendations for the safe operation of HCUs.

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Conflict of interest

The authors have shared the manuscript with the manufacturer before publication. This has not led to changes of the content. The authors have declared that they have no competing interests.

Authors' contributions

SH, MAS, OH and TE were part of the outbreak team at RKI and conducted the epidemiological outbreak investigations. SH, TE and DP designed the investigation. SH, TE, DP, AJ, DLM and CH drafted the manuscript. All authors critically revised the manuscript and approved the final version. TE is corresponding author and guarantor.

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Persisting transmission of carbapenemase-producing *Klebsiella pneumoniae* due to an environmental reservoir in a university hospital, France, 2012 to 2014

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In France, the proportion of episodes of carbapenemase-producing *Enterobacteriaceae* (CPE) with no recent stay or hospitalisation abroad is increasing. In this study, we investigate epidemiological links between apparently unrelated cases of OXA-48-producing *Klebsiella pneumoniae* (Kp OXA-48) colonisation or infection. We genotyped detected organisms by repetitive sequence-based PCR, and used a dynamic registry of cases and contacts to cross-reference patients' hospital stays. Between 1 November 2012 and 28 February 2014, 23 Kp OXA-48 cases were detected in a university hospital in Montpellier, of which 15 were involved in three outbreaks: outbreaks I and II occurred in November 2012 and outbreak III in October 2013. Molecular comparison of bacterial strains revealed clonal identity between cases involved in outbreaks II and III and four single cases. Cross-referencing of hospital stays revealed that these single cases and the index case of outbreak III had occupied the same room. Active case search among former occupants of that room found an additional Kp OXA-48 carrier. A clonal strain was isolated from the sink of that room. The epidemiological link between the contaminated room and outbreak II remained undetected. This study is a reminder that environmental reservoirs should be considered as a source of CPE transmission.

Introduction

Since the 2000s, rates of carbapenemase-producing *Enterobacteriaceae* (CPE) have increased worldwide [1] and become endemic in several European countries [2]. *Enterobacteriaceae* cause various infections (urinary tract, digestive or respiratory infections) and the presence of carbapenemase increases mortality rates [3,4]. In France, where CPE are still considered emergent and mostly imported from Mediterranean countries, no link with a foreign country (hospitalisation or travel abroad of the index case) was reported for half (819/1,625) of the events (defined as one or more epidemiologically related CPE cases) notified by infection control teams and/or laboratories between January 2004 and March 2015 [5]. The most frequently found CPE in France is OXA-48-producing *Klebsiella pneumoniae* (Kp OXA-48) and in 2014, 656 episodes were notified [5].

In our healthcare facility, a teaching hospital in southern France, three outbreaks of Kp OXA-48 infections and colonisations occurred in November 2012 and October 2013 and several single cases occurred in 2013. While one of these single cases was imported from North Africa, the remaining could not be linked to an epidemiological source, raising the question of unidentified bacterial reservoirs either within our hospital or circulating in the community. The aim of this study was to investigate epidemiological links between Kp OXA-48-positive patients, with no evident epidemiological source of transmission and seemingly unrelated, that occurred in our facility between 1 November 2012 and 28 February 2014.

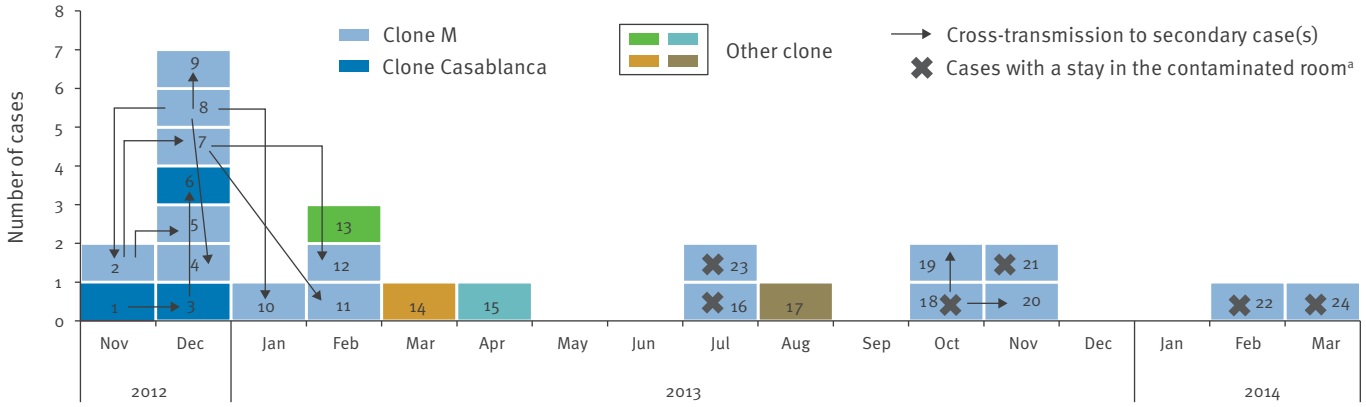
Methods

Setting

The study was conducted in the University Hospital of Montpellier, a 2,634-bed tertiary care teaching hospital, organised in five distinct hospital sites. It has seven intensive care units (ICU), including a 12-bed neurosurgical ICU. The Infection Control (IC) team comprises 1.6 full-time doctors, seven nurses and an attached IC

Figure 1

Epidemic curve of OXA-48-producing *Klebsiella pneumoniae*, University Hospital of Montpellier, France, 1 November 2012–28 February 2014 (n = 24)



One square represents one case. Cases are numbered by order of inclusion in the investigation. Each colour represents a different clone of OXA-48-producing K. pneumoniae.

[a] One intensive care unit room where OXA-48-producing K. pneumoniae was isolated from environmental samples.

laboratory. Clinical wards are regularly visited to evaluate healthcare professionals' compliance with standard precautions, hand cleaning and hospital hygiene. In January 2014, a dynamic registry of CPE cases and contacts (an ongoing Excel file) [6] was set up to facilitate case management, contact tracing and alert upon readmission of cases or uncleared contacts (incompletely screened contacts, see study definitions). All CPE cases and contacts diagnosed in our hospital from October 2012 onwards were retrospectively registered, and all incident cases and contacts thereafter.

Multidrug-resistant organism surveillance policy (implemented in 2006)

According to French recommendations, all patients with more than 48 hours continuous stay in the ICU undergo active screening (weekly nasal and rectal swabs) for multidrug-resistant organisms (MDRO). In other units, screening is performed on patients presenting risk factors (history of previous MDRO carriage, transfer from a long-term care facility, chronic wounds and/or indwelling medical device). Since 2013, in response to national recommendations [7], patients transferred from a foreign hospital or with a history of hospitalisation abroad in the previous 12 months have been screened for MDRO and CPE upon admission. A daily automatic report from the microbiology laboratory informs the IC team of prevalent MDRO-positive clinical or screening samples.

Hospital hygiene and environmental control policy

Nursing auxiliaries trained in procedures written by the IC team carry out the cleaning of patients' rooms. The protocols include daily disinfection of sinks with bleach solution at a concentration of 0.5% of available chlorine, with at least one hour of contact.

Environmental surveillance is performed by the IC laboratory and involves regular screening of sinks on high-risk wards and sinks on any ward with a history of contamination. Each ICU sink is screened twice a year by sampling tap water and tap and trap surfaces. A more comprehensive sampling of dry and damp surfaces is performed during outbreaks for the detection of potential reservoirs.

Study definitions

Cases of Kp OXA-48 were defined as patients (infected or colonised) identified in our facility between 1 November 2012 and 28 February 2014, with a Kp OXA-48-positive culture from any site during their hospitalisation. An outbreak was defined as at least two cases linked by an epidemiological chain of transmission: an index case followed by one or more hospital-acquired secondary cases, with indistinguishable bacterial strains according to molecular biology. A sporadic case was defined as a single case, or the index case of a cluster, that couldn't be linked to an epidemiological source.

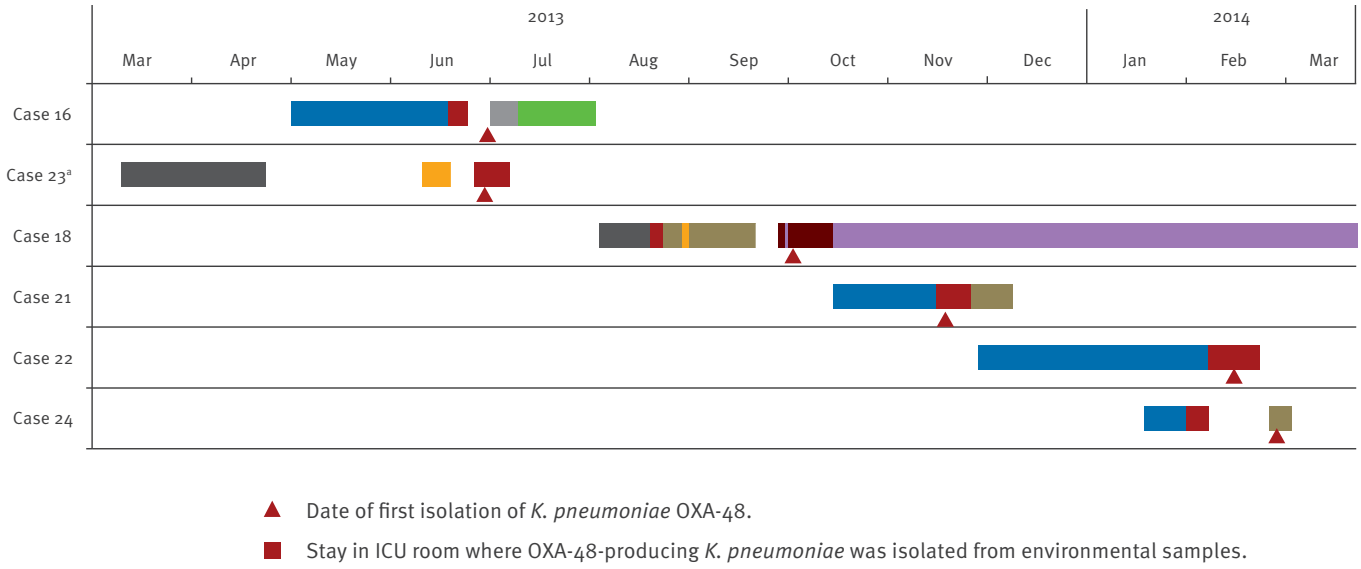
Contacts were the patients cared for by the same healthcare team as a case. Their screening (repeated weekly rectal or stool swabbing) was followed up until three negative results.

Microbiological studies

Clinical strains were isolated during routine practice of medical microbiology according to clinical laboratory policy. Briefly, detection of CPE was performed using a combination of different media to screen for OXA-48 and other CPE (chromID CARBA SMART, bioMérieux, France). The resistance profile was interpreted according to the recommendations of the Antibiogram Committee of the French Microbiology Society (CA-SFM).

Figure 2

Synoptic curve of sporadic cases with the same clone of OXA-48-producing *Klebsiella pneumoniae*, University Hospital of Montpellier, France, March 2013–March 2014 (n = 6)



Each colour represents a different ward.

[a] Patient identified retrospectively because of an initially mistaken identity.

When suspected from selective media and resistance profile, the presence of the carbapenemase gene was confirmed by the regional reference laboratory (Nîmes University Hospital) using the Check-MDR CT102 microarray (Check-Points, the Netherlands). Bacterial strains were compared by in-house repetitive sequence-based PCR (rep-PCR) [8].

Environmental samples (surfaces and sinks) were taken with sterile, cotton-tipped swabs. After a specific search for *Enterobacteriaceae* on selective medium (Mac Conkey Agar), matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) mass spectrometry was performed for identification.

Results

Characteristics of cases

Between 1 November 2012 and 28 February 2014, 24 Kp OXA-48-positive patients were identified in the University Hospital of Montpellier. Their epidemiological characteristics are shown in Table 1.

Two outbreaks occurred in November 2012 (outbreaks I and II) and one in October 2013 (outbreak III); they involved three, nine and three cases, among which 12 were hospital-acquired secondary cases (Figure 1). Cases are numbered by order of discovery in the course of the investigation. Case 23 was included later than the discovery date, in spite of an early positive Kp OXA-48 finding, because of a mistaken identity at the regional laboratory.

Outbreaks I and II happened simultaneously (indeed, the second one was revealed through contact tracing of the first), and involved two distinct bacterial clones in rep-PCR (data not shown). Outbreak I occurred from an index case (case 1) transferred from a Moroccan hospital (clone Casablanca) and generated two secondary cases (cases 3 and 6); in outbreak II, clone M was found in nine patients (cases 2, 4, 5, 7 to 12) and stemmed from an index case (probably case 8) with no known source of contamination.

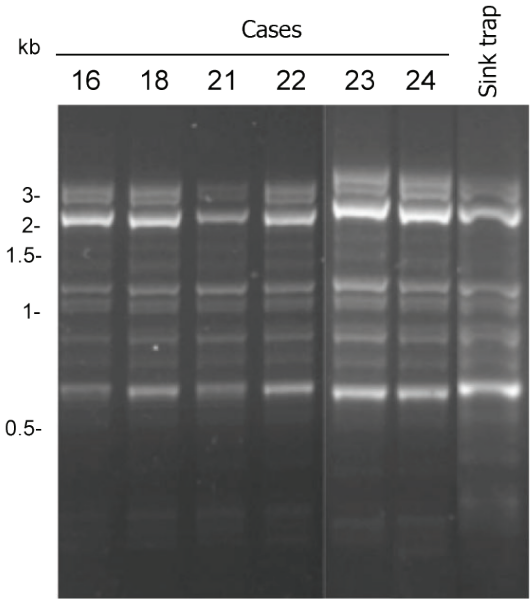
Seven sporadic cases of Kp OXA-48 (cases 13 to 18 and 21) were identified in 2013: six remained single cases and one (case 18) was the source of outbreak III (two secondary cases, cases 19 and 20). Among these sporadic cases, only one (case 13) had a history of healthcare in a foreign country. For the six others, no contact with a known CPE carrier was found and three had previously negative MDRO screenings. A recent hospitalisation or residency in a long-term healthcare facility was found for three of the six cases and no significant history was found for the three other cases.

In February 2014, a new case (case 22) was diagnosed in the neurosurgical ICU, also seemingly unrelated to any source of contamination. At the same time, we were informed by the regional laboratory that a misidentified case from July 2013 was to be considered (case 23). By February 2014, a total of nine sporadic cases were under investigation.

A comparison of all the bacterial strains was performed by rep-PCR in February 2014 (data not shown).

Figure 3

In-house rep-PCR profiles obtained for clinical (n = 6) and environmental (n = 1) *Klebsiella pneumoniae* strains belonging to clone M, France, 1 November 2012–28 February 2014



The origin of the strain is indicated at the top of the gel. The nomenclature of strains follows the numbering of the cases. Sizes in kb corresponded to 1 kb ladder.

It showed that the three cases involved in outbreak III also belonged to clone M identified in outbreak II. More surprisingly, it also revealed that four of the single cases (cases 16, 21, 22 and 23) shared that same clone M profile. Overall, clone M was found in 16 cases: nine from outbreak II, three from outbreak III and four single cases. The clone Casablanca was not identified in other than the three cases of outbreak I; four different clones were diagnosed in the remaining four single cases (cases 13, 14, 15 and 17).

Epidemiological investigation

Using the registry of CPE cases and contacts, cross referencing of the cases' hospital stays highlighted that four of the sporadic cases (cases 16, 18, 21 and 23) had occupied the same room in the neurosurgical ICU before detection of their Kp OXA-48, following one another at intervals of two to 84 days between June and December 2013 (Figure 2). All four were colonised with the epidemic clone M. The patient present in that room at the time of investigation, in February 2014, also turned out to be colonised by Kp OXA-48 (case 22). Retrospective case search among patients admitted to this room in the three months before the investigation detected one additional case (case 24).

In all, six cases with clone M had been hospitalised in this ICU room between June 2013 and February 2014. Five of these cases were men and their median age was 43 years (range: 23–51); their underlying conditions were severe traumatic head or spine injury (n = 4) or haemorrhagic cerebrovascular events (n = 2). Kp OXA-48 was isolated from a rectal swab in four of these cases and from tracheal aspiration in the other two. All six patients were considered as colonised and none received antibiotic treatment for a clinical infection involving the epidemic bacterial strain. No other epidemiological link was found between these six cases, and no contact was found between them and the cases of outbreak II.

Environmental investigation

Thirty-nine swabs were taken on different dry surfaces and five on damp surfaces of the involved ICU room on 21 and 25 February 2014 (while the room was occupied by case 22). The room had a single bed and a hand washing sink. Two samples from the siphon and the tap aerator of the water outlet yielded Kp OXA-48. This bacterium was not detected on the dry surfaces of the room, the nursing station or adjacent bedrooms. Comparison of environmental strains with the six patients who had occupied the room showed identical pulsed-field gel electrophoresis (PFGE) profiles (Figure 3). Thorough cleaning and surface disinfection were performed and new sink trap and tap were installed; extensive environmental sampling performed in March 2014, after the intervention (total: 55 samples), did not find Kp OXA-48. No additional sporadic case was identified after implementation of the environmental measures.

Discussion

We report here the persistent transmission of a single Kp OXA-48 clone and provide arguments in favour of a role of moist environments in the transmission of CPE. Water and water outlets are well-reported reservoirs for nosocomial transmission of *Pseudomonas aeruginosa* [9,10], and the risk of acquiring multidrug-resistant (MDR) bacteria from prior room occupants in ICU has been demonstrated for MDR *Acinetobacter baumannii, P. aeruginosa* [11] and organisms such as meticillin-resistant *Staphyloccocus aureus* and vancomycin-resistant enterococci [12]. The role of an environmental source in the transmission of extended spectrum beta-lactamase-producing (ESBL) *Enterobacteriaceae* [11,13] has been underestimated in spite of outbreak reports supporting the evidence [14,15]. As for CPE outbreaks, patient-to-patient cross-transmission is the privileged hypothesis, supported by numerous reports of negative environmental investigations [16-20]. However, a few outbreaks with environmental transmission of CPE have been described in Australia, Spain and Norway [21-23]; these protracted outbreaks (20 to 30 months duration) occurred in ICUs between 2007 and 2012. A recent meta-analysis has established that the risk of MDRO acquisition from prior occupants is as important for Gram-negative as for Gram-positive organisms [24].

Table

Epidemiological characteristics of OXA-48-producing *Klebsiella pneumoniae* cases diagnosed in Montpellier University Hospital, France, 1 November 2012–28 February 2014 (n = 24)

Characteristics	Cases
Sex ratio male/female	1.7
Age in years, median (min–max)	62 (23–85)
Link with a foreign country in the previous 12 months (hospitalisation abroad)	2
Cases involved in outbreaks	15
Index cases	3
Secondary cases	12
Single cases	9
Length of stay in days in a hospital unit[a] , median (min–max)	11 (1–104)
Clinical infections	4

[a] Only stays in the Montpellier University Hospital are considered.

Transmission of microorganisms from a contaminated sink trap to patients is commonly attributed to splashing [25], either directly on the patient or onto healthcare professionals' hands. It has been reported that hospital room design is a key element in environmental contamination by MDRO [15,25]. It has also been suggested that rates of environmental contamination are higher for EBSL *K. pneumoniae* than EBSL *Escherichia coli* [26,27].

In our study, despite the daily chlorination process, the epidemic clone was identified from the siphon of the sink in room occupied by a Kp OXA-48-colonised patient. The direction of the contamination can be questioned (the positive patient could have contaminated the sink) and it was not possible to determine the origin of the environmental strain. However, there are indirect arguments in favour of a sink-to-patient contamination route. Firstly, this patient had prior negative MDRO screenings before their stay in this room and was otherwise unrelated to the other cases with the same clone. The same was true for the case retrospectively detected among prior occupants of the room. Secondly, no further case acquired in our hospital has been identified after the corrective works on the incriminated water outlet.

We were not able to establish the transmission link between the patients sharing the ICU room and outbreak II (involving the same bacterial clone). Other cases may have gone undetected among prior occupants of the room, as we did not call them all back for extensive screening. Furthermore, a study carried out from February 2011 to February 2013 in our region found a clonal diversity among Kp OXA-48 strains identified in the region [28], and the circulation of a community strain with the same PCR profile seems unlikely. Hence, the hypothesis of a missing link in the nosocomial transmission chain remains unresolved.

In our study, molecular epidemiology proved a useful complement to classical investigation methods. Indeed, a transmission link between the cases was not straightforward, as they were not grouped in time and space when their first CPE-positive culture was known. The molecular findings prompted a thorough investigation of these apparently unrelated sporadic cases and revealed an unsuspected environmental reservoir. Even if cross-transmission remains the privileged hypothesis when investigating a CPE outbreak, as rates of Kp-OXA 48 cases increase in our hospitals, our study reminds us to consider environmental reservoirs as a source of CPE transmission.

Conflict of interest

None declared

Authors' contributions

Conception and design: A. Lotthé/S. Parer/B. Clarivet; acquisition of data: B. Clarivet/D. Grau/E. Jumas-Bilak/H. Jean-Pierre, analysis and interpretation: B. Clarivet/E. Jumas-Bilak/A. Pantel, redaction: B. Clarivet / A. Lotthé, final approval of the version to be published: all the authors.

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Marked increase in leptospirosis infections in humans and dogs in the Netherlands, 2014

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In the Netherlands, 97 human leptospirosis cases were notified in 2014. This represents a 4.6-fold increase in autochthonous cases (n = 60) compared with the annual average between 2010 and 2013. Most cases had symptom onset between June and November. This marked increase in humans coincided with an increase of leptospirosis in dogs. In 2014, 13 dogs with leptospirosis were reported, compared with two to six dogs annually from 2010 to 2013. The majority of the autochthonous cases (n = 20) were linked to recreational exposure, e.g. swimming or fishing, followed by occupational exposure (n = 15). About sixty per cent (n = 37) of the autochthonous cases were most likely attributable to surface water contact, and 13 cases to direct contact with animals, mainly rats. A possible explanation for this increase is the preceding mild winter of 2013–2014 followed by the warmest year in three centuries, possibly enabling rodents and *Leptospira* spp. to survive better. A slight increase in imported leptospirosis was also observed in Dutch tourists (n = 33) most of whom acquired their infection in Thailand (n = 18). More awareness and early recognition of this mainly rodent-borne zoonosis by medical and veterinary specialists is warranted.

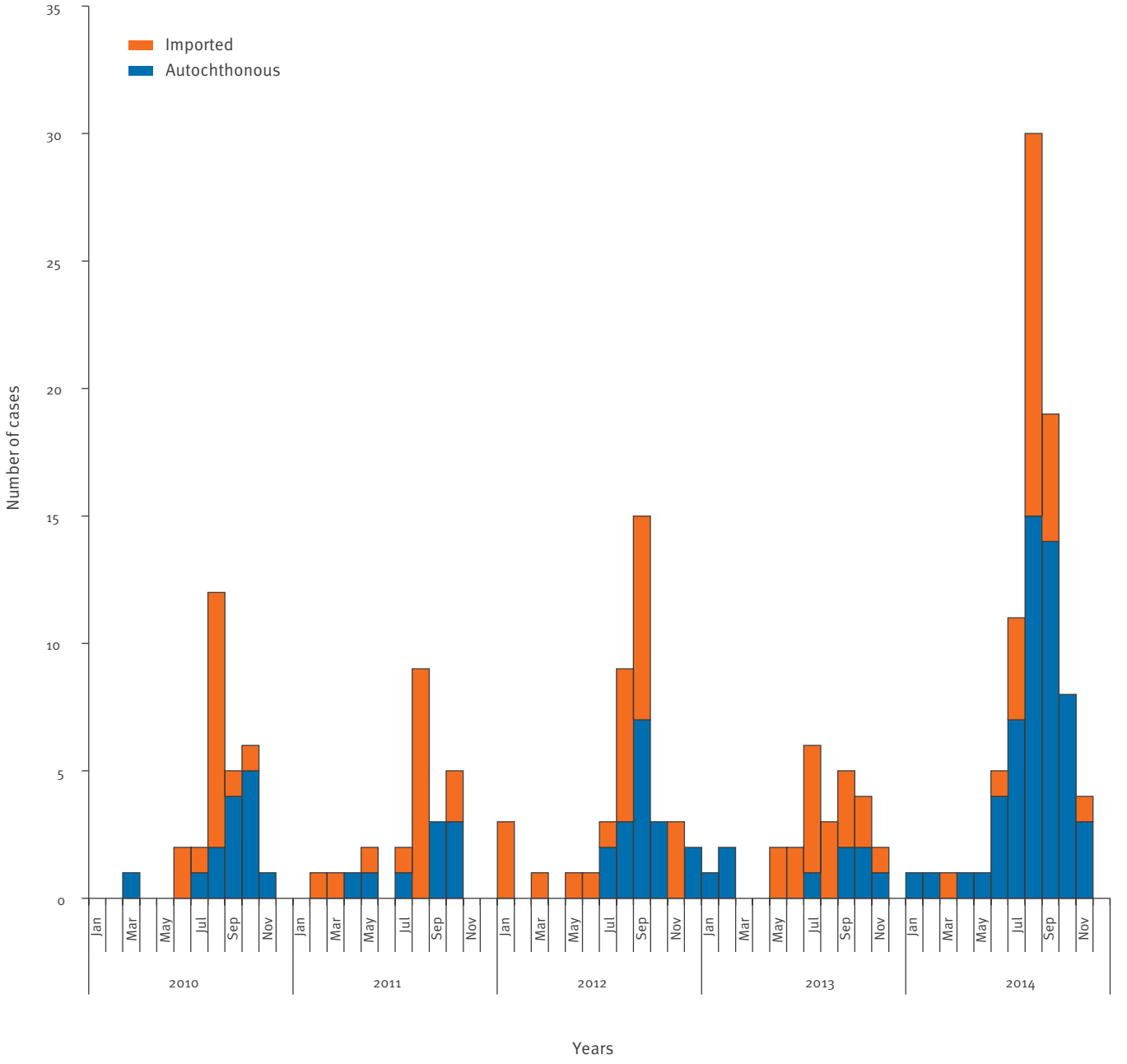
Background

Leptospirosis is a zoonosis caused by pathogenic *Leptospira* species (spp.) and may result in a broad clinical spectrum of disease, ranging from asymptomatic infections to severe disease manifestations known as Weil's syndrome, characterised by the triad of jaundice, acute renal failure and bleeding manifestations, and severe pulmonary haemorrhage syndrome (SPHS) with a high case–fatality rate [1-3]. Transmission to humans usually occurs via direct or indirect contact with urine of infected animals. A wide variety of animal species, primarily mammals such as rodents, cattle and dogs, may serve as a reservoir of leptospires [1]. The usual port of entry is the skin via abrasions or cuts but infection may also occur via the conjunctiva [2]. In dogs, leptospirosis can cause severe, life-threatening infections with vascular damage, liver and renal failure. Pulmonary symptoms have recently been reported as well [4]. There are nearly 300 pathogenic *Leptospira* serovars, often specific to particular host reservoirs, belonging to 29 serogroups, and therefore an indication for the most likely source of human infections [2].

In the Netherlands, leptospirosis has been a mandatory notifiable disease in humans since 1928 [5]. It mainly occurs as a sporadic disease and is primarily caused by two serogroups of *Leptospira* spp.: Icterohaemorrhagiae (serovars Icterohaemorrhagiae and Copenhageni) with rats as reservoir and Grippotyphosa (serovar Grippotyphosa type Duyster) with mice as reservoir. In animals, only leptospirosis caused by *Leptospira borgpetersenii* serovar Hardjo is a notifiable disease. In the late 1980s and early 1990s, dairy cattle were a major source of serovar Hardjo [6]. Due to an effective control and monitoring programme in the 1990s, serovar Hardjo became rare in Dutch cattle [7], resulting in a marked decrease in autochthonous human dairy farm fever (Hardjo) cases [8]. Since 2000, approximately 30 human leptospirosis cases have been diagnosed annually in the Netherlands, mostly associated with recreational exposures [6,9]. Leptospirosis has an annual peak incidence occurring in late summer and autumn in temperate regions like the Netherlands [2]. Due to increasing globalisation, the proportion of imported human cases has gradually increased over time. Most cases acquired leptospirosis outside Europe, mainly in countries in south-east Asia [6].

Figure 1

Autochthonous (n = 60) and imported cases (n = 33) of leptospirosis by month of illness onset, the Netherlands, 2010–2014



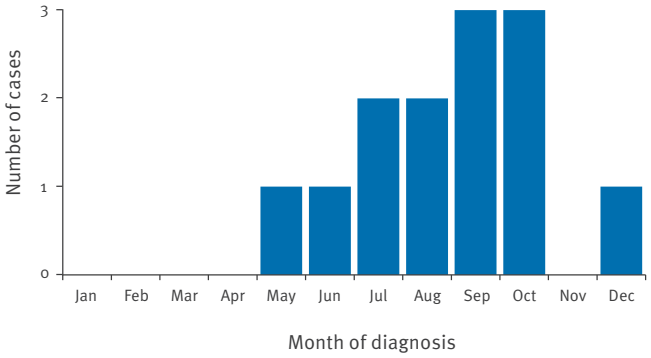
In September 2014, an increase in notified leptospirosis cases was observed by the National Leptospirosis Reference Centre (NRL), which alerted the National Institute of Public Health and the Environment (RIVM) as part of their national reference tasks. The NRL, which is also World Health Organization (WHO)/Food and Agriculture Organization of the United Nations (FAO)/World Organisation for Animal Health (OIE) Collaborating Centre for Reference and Research on Leptospirosis, shared this alert with the WHO Collaborating Centre on Leptospirosis in France, which, in turn, confirmed a coinciding increase in leptospirosis in mainland France. They posted their joint findings in an urgent inquiry in the Epidemic Intelligence Information System (EPIS) for Food and Waterborne Diseases of the European Centre for Prevention and Control (UI-272, EPIS) on 31 October 2014. An increase in confirmed leptospirosis in dogs and inquiries by veterinarians about suspected cases was noted by the Dutch Veterinary Microbiological Diagnostic Center in October 2014. In this report, we have combined all available data to describe this marked increase in leptospirosis infections in humans and dogs, and provide case characteristics such as symptoms, travel history, possible sources of exposure and serogroup information.

Figure 2

Geographical distribution of autochthonous (n = 60) and imported cases (n = 33) based on postal code of residence, the Netherlands, 2014

Figure 3

The number of dogs diagnosed with leptospirosis by the Veterinary Microbiological Diagnostic Center, by month of diagnosis, the Netherlands, 2014



Methods

We used surveillance reports stored in the national surveillance database at the National Institute for Public Health and the Environment (RIVM). Clinicians and general practitioners send clinical specimens of patients suspected for leptospirosis to the National Leptospirosis Reference Centre (NRL) for laboratory evaluation using microscopic agglutination test (MAT) and an in-house-developed IgM-ELISA for diagnostic confirmation based on detection of antibodies. When patient serum is collected before the 11th day after date of symptom onset, tests to detect leptospiral antigen (culture and PCR) are performed as well; PCR is always performed on urine because leptospiral DNA can be detected in urine at all stages of the disease. The presumptive serogroup was deduced from the highest MAT titre with a pathogenic serovar in a follow-up sample. A case of leptospirosis is considered confirmed positive for *Leptospira* when positive by culture and/or PCR and/or serology (MAT or IgM ELISA) and has fever or at least two of the following symptoms: rigors, headache, myalgia, running eyes, bleeding in skin and mucosa, rash, jaundice, myocarditis, meningitis, renal failure or pulmonary haemorrhagic symptoms.

Patients with confirmed leptospirosis are reported by the NRL to the Municipal Health Service (MHS) that collects case characteristics, performs source tracing and, if needed, instigates control measures [3]. Detailed travel history in the month before date of symptom onset and the most likely source of infection to determine whether a case is classified as autochthonous or imported. The MHS notifies each laboratory-confirmed case that adheres to the clinical case definition to the national surveillance database at the RIVM [3].

The MHS also notifies autochthonous cases to the Dutch Food and Consumer Product Safety Authority (NVWA) if site investigation is necessary, for instance if a petting farm is suspected as source of human infection [10]. GD Animal Health, which implemented a nationwide system for animal health surveillance 2003, notifies the NVWA when GD Animal Health test bulk milk from dairy herds or (slaughterhouse) blood samples from non-dairy herds positive for *Leptospira* spp. using ELISA. The NVWA then performs source investigation.

For this study, we compared all notified leptospirosis cases in 2014 with diagnosed patients in the NRL patient database based on birth year, sex and four-digit postal code, for completeness and confirmation of serogroup details and laboratory method. Case characteristics such as date of symptom onset, symptoms, travel history, relevant exposures and serogroup information were analysed. Diagnostic delay is defined as the median time period between day of symptom onset and laboratory confirmation by NRL.

The Veterinary Microbiological Diagnostic Centre (VMDC) receives sera from dogs in the Netherlands showing clinical signs of leptospirosis, which are confirmed by a combination of IgM and IgG-ELISA [11]. No information is available about the infecting serogroups in dogs. The VMDC also acts as an information desk for Dutch veterinary practitioners treating dogs suspected to have leptospirosis, and all phone calls are registered. These data were used to analyse the occurrence of leptospirosis in dogs in the Netherlands.

Table 1A

Characteristics of autochthonous (n = 60) and imported (n = 33) leptospirosis cases, the Netherlands, 2014

Characteristics	Autochthonous	Imported
Male sex	49	26
Median age in years (range)	48 (10–75)	42 (13–64)
Region		
North	9	1
West	28	24
East	20	5
South	2	3
Other [a]	1	0
Most likely type of exposure		
Recreational	20	29
Swimming	10	12
Fishing	5	0
Water sports	2	8
Water contact [b]	3	9
Occupational	15	0
Farmer	6	0
Dredging	2	0
Rat catcher	1	0
Gardener	1	0
Handyman	1	0
Kite surf instructor	1	0
Water management	1	0
Sheet piling	1	0
Police trainee	1	0
Residential	12	-
Gardening	3	-
Rat/mice presence around home	3	-
Cleaning pond	2	-
Pet mice	1	-
Water/mud	1	-
Not specified	2	-
Accidental	7	NA
Fell in water	4	NA
Rodent bite	3	NA
Not specified	7	4
Most likely route of infection		
Surface water	37	29
Ditch	9	0
Lake	9	4
Canal/river	7	9
Pond	2	0
Indoors	2	0
Unknown	8	16
Animal	13	0

NA: not available; -: not applicable.

[a] Not a Dutch resident

[b] Multiple types of water contact, or type of water contact not further specified

Results

Humans

In 2014, a total of 97 human cases (incidence 0.57/100,000 inhabitants) were notified in the Netherlands (Figure 1, Table 1). Twenty-five cases tested positive based on serology and culture or PCR. Thirty-three cases tested positive for culture or PCR and 39 cases only had positive serology. The majority of these cases (60/97) were autochthonous as they most likely contracted the infection in the Netherlands, representing a 4.6-fold increase compared with 2010–2013. Most of them became symptomatic between June and November, with a peak in August. The rise was one month earlier compared with the years from 2010 to 2013. A 1.6-fold increase (33/97) in imported cases was also observed. Country of infection was unknown for four cases. The median age was 48 years (range: 10–75 years) and 42 years (range: 13–64 years) for autochthonous cases and imported cases, respectively. The majority of autochthonous (49/60) and imported cases (26/33) were male. Autochthonous cases occurred sporadically based on the four-digit postal code of their residential address and were mainly resident in the western (28/60) and eastern (20/60) regions of the Netherlands. A small proportion was resident in the northern (9/60) and southern (2/60) regions (Figure 2). Imported cases were mainly resident in the agglomerated western region (24/33) of the Netherlands.

Symptoms and hospitalisation

Among cases for whom symptoms were reported, fever was the most frequently reported symptom (79/86). Other symptoms reported were, in order of prevalence, myalgia, headache, rigors, renal failure, jaundice (Table 2). Autochthonous cases more often presented with renal failure, jaundice and haemorrhagic symptoms compared with imported cases. Meningitis was reported in one autochthonous case and myocarditis in one imported case. Fifty-four of 60 of the autochthonous and 23/33 of the imported cases were hospitalised. No deaths were reported. The diagnostic delay was 15 days (range: 3–50 days) for autochthonous cases and 12 days (range: 3–49 days) for imported cases. From 2010 to 2013, the diagnostic delay was 14 days (range: 5–64 days) for autochthonous cases and 21 days (range: 3–84 days) for imported cases.

Serogroups

Among the autochthonous cases, 26/60 cases allowed the presumptive deduction of the infecting serogroup based on MAT titres: Icterohaemorrhagiae (9/26), Grippotyphosa (8/26), Javanica (3/26), Sejroe/Hebdomadis/Mini complex (2/26), Sejroe (2/26), Mini (1/26) and Pomona (1/26). Among imported cases, the presumptive serogroup could be deduced for 8/33 cases: Australis (2/8), Celledoni (2/8), Sejroe (1/8), Mini (1/8), Icterohaemorrhagiae (1/8) and Cynopteri (1/8). For the remaining 59 cases, the serogroup could not be determined, mostly because no follow-up serum sample was received.

Table 1B

Characteristics of autochthonous (n = 60) and imported (n = 33) leptospirosis cases, the Netherlands, 2014

Characteristics	Autochthonous	Imported
Most likely route of infection		
Rat	8	0
Mouse	2	0
Cow	1	0
Not specified	2	0
Soil	4	4
Unknown	6	0
Rat presence reported		
Yes	21	NA
No	18	NA
Not reported	21	NA
Serogroup	n=26	n=8
Icterohaemorrhagiae	9	1
Grippotyphosa	8	0
Javanica	3	0
Sejroe/Hebdomadis/Mini	2	0
Sejroe	2	1
Mini	1	1
Pomona	1	0
Australis	0	2
Cynopteri	0	1
Celledoni	0	2

NA: not available; -: not applicable.

Country of infection

Imported cases mainly acquired leptospirosis in countries in south-east Asia, of which 18/33 in Thailand. Other countries were Cuba (three cases), Cambodia and Sri Lanka (two cases each), Indonesia, Laos, Malaysia, Nepal, Costa Rica, Guatemala, Suriname and France (one case each).

Transmission route and presence of rodents

Autochthonous cases mainly acquired leptospirosis during recreational activities (20/60) such as swimming (10/20) and fishing (5/20), followed by occupational activities (15/60), mostly observed among farmers (6/15). Cases also contracted leptospirosis during activities at their place of residence (12/60) such as gardening (3/11), and due to accidents (7/60), which included patients who fell in water (4/7) or were bitten by a mouse (3/7). About two-thirds (37/60) of the autochthonous cases were most likely attributable to surface-water contact, including contact with water in ditches (9/37), lakes (9/37), canals/rivers (7/37), ponds (2/37), indoor surface water (e.g. water in basement) (2/37). Direct animal contact (13/60), including rats (8/13), mice (2/13) and cows (1/13), and soil contact (4/60) were also reported. Around one-third (21/60) reported having seen rats or mice at the location where they most probably acquired the infection. Imported cases were almost all attributable to contact with surface water (29/33) and contracted the disease during recreational activities (29/33) such as swimming (12/29) or other water sports (8/29).

Source investigations based on notified human cases

The NVWA received 26 notifications of autochthonous cases in 2014, mostly from a MHS, accompanied by a request for animal source investigation. For nine notifications, site investigations were performed, and if necessary, animal or environmental samples were collected. In two site investigations, animal samples were found positive for *Leptospira* antibodies.

In August 2014, serovar Hardjo was identified in a Dutch farmer. He was most likely infected by his dairy cattle because his bulk milk had previously tested positive by GD Animal Health for the presence of *Leptospira* antibodies using ELISA. Investigation by the NVWA revealed that this cattle herd most likely acquired the infection via German cattle, since they accidently grazed on the same pasture at the same time.

The second source investigation included a carp farmer, positive for leptospirosis in November, who reported a rat infestation at his farm. A captured rat tested by the NRL was PCR-positive. Culture and further characterisation was not successful, but the PCR melting curve results of the farmer and rat samples were similar and matched with *L. interrogans*.

Dogs

The VMDC reported 13 dogs with leptospirosis in 2014, mostly diagnosed between June and October (Figure 3). From 2010 to 2013, two to six dogs were diagnosed annually according to VMDC. The number of inquiries on suspected leptospirosis in dogs doubled in 2014 (n = 54) compared with 2013 (n = 24).

Discussion

A marked increase in autochthonous cases of leptospirosis was observed in the Netherlands in 2014, particularly during the second half of the year, from June until November, resulting in one of the highest incidence rates in Europe [12].

Cases mainly acquired leptospirosis during recreational activities such as swimming and fishing, in contrast with other western European countries, where autochthonous leptospirosis infections are predominantly associated with occupational activities [13-15]. A possible explanation for the increase of autochthonous cases is the preceding mild winter of 2013 to 2014 followed by the warmest year in three centuries in Europe [16,17], possibly enabling rodents and also excreted *Leptospira* to better survive [2,18,19]. Warm weather might also be related to increased outdoor recreational activities due to the early high temperatures in spring 2014, leading

Table 2

Main symptoms, hospitalisation and median diagnostic delays of autochthonous (n = 60) and imported (n = 33) leptospirosis cases, the Netherlands, 2014

	Autochthonous (n = 56)	Imported (n = 30)
Symptoms[a]		
Fever	51	28
Myalgia	35	21
Rigors	31	17
Headache	26	25
Renal failure	21	6
Jaundice	17	4
Skin rash	8	5
Hospitalisation		
Yes	54	23
No	4	7
Unknown	2	3
Median diagnostic delay in days (range)	15 (3–50)	12 (1–49)

[a] Multiple answers were possible

to more exposure, and an earlier seasonal rise in cases than the normal seasonal trend [20]. The increase in autochthonous cases supports a recent French study [13] hypothesising an increase in leptospirosis burden in European countries due to global warming, increasing populations of urban rodents or other animal reservoirs [21], human population growth, urbanisation and increasing international travels. Germany also noted a similar increase in autochthonous cases in 2014, which they likewise attributed to a warm and humid climate [22]. In the Netherlands, the number of imported cases was also elevated, but to a lesser extent. This might be due to increased awareness of leptospirosis in Dutch travellers among medical specialists, indicated by the decreased diagnostic delay compared with 2010 to 2013.

In 2014, serogroup Sejroe/Hebdo/Mini complex was identified in two autochthonous cases in the Netherlands, which is remarkable because this serogroup had only been identified in one previous autochthonous case in 1998 [6]. One of the cases acquired leptospirosis after being bitten by a mouse that was intended for feeding to a snake, and the other case had multiple possible sources of infection. For the first time in 16 years, serovar Hardjo was identified in a dairy cattle farmer in the Netherlands. This was surprising, because 99% of the dairy and beef cattle farms in the Netherlands had a Hardjo-free status in 2014 [7]. However, source investigations revealed that the case most likely acquired the infection via German cattle, in which serovar Hardjo is common [14].

Also remarkable, although based on small numbers, is the concomitant increase in canine cases in the second half of 2014, strengthening the hypothesis of increased environmental exposure. A monitoring programme in rodents begun in 2014 revealed that *Leptospira* are present and widespread in the rat population in the Netherlands (data not shown, personal communication, Joke van der Giessen, December 2014).

A major limitation of this study was the use of passive human surveillance data likely reflecting the more severe hospitalised cases, which leaves milder cases often unrecognised [1,23,24]. This should be taken into account when interpreting the clinical presentation of cases described in this article. Also the number of canine leptospirosis cases is likely to have been underestimated, as it depends on the veterinary clinicians' ability to identify leptospirosis in dogs. Unfortunately, the infecting serogroup based on MAT titres could only be presumed in less than half of the cases, because follow-up samples were often not received.

The results suggests that prevention efforts should be aimed at advising the general public and high risk occupational groups that have direct or indirect contact with rat or mouse urine about possible precautions to reduce exposure to *Leptospira*. In the future, monitoring programmes in rodents should focus on predicting risk of zoonotic transmission and developing preventive strategies [9]. Furthermore, vaccination of dogs should be promoted in the Netherlands, where currently only around 55% of dogs are vaccinated [9]. Preventive measures are generally advisable when a dog is suspected for leptospirosis. More awareness and early recognition of this mainly rodent-borne zoonosis by medical specialists is warranted.

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Conflict of interest

There are no competing interests for any of the authors.

Authors' contributions

Roan Pijnacker conceptualised, drafted and revised the manuscript as submitted; Marga G.A. Goris provided laboratory data on human leptospirosis cases, and critically reviewed and revised the manuscript as submitted; Margreet J.M. te Wierik conceptualised, reviewed and revised the manuscript as submitted; Els M. Broens provided data on leptospirosis in dogs, and critically reviewed and revised the manuscript as submitted; Joke W.B. van der Giessen provided data on rodent monitoring, and critically reviewed and revised the manuscript as submitted; Mauro de Rosa provided data on site investigations, and critically reviewed and revised the manuscript as submitted; Jaap A. Wagenaar critically reviewed and revised the manuscript as submitted; Rudy A. Hartskeerl: citically reviewed and revised the manuscript as submitted; Daan W. Notermans critically reviewed and revised the manuscript as submitted; Kitty Maassen critically reviewed and revised the manuscript as submitted and Barbara Schimmer conceptualised, reviewed and revised the manuscript as submitted.

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Best practices in ranking communicable disease threats: a literature review, 2015

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The threat of serious, cross-border communicable disease outbreaks in Europe poses a significant challenge to public health and emergency preparedness because the relative likelihood of these threats and the pathogens involved are constantly shifting in response to a range of changing disease drivers. To inform strategic planning by enabling effective resource allocation to manage the consequences of communicable disease outbreaks, it is useful to be able to rank and prioritise pathogens. This paper reports on a literature review which identifies and evaluates the range of methods used for risk ranking. Searches were performed across biomedical and grey literature databases, supplemented by reference harvesting and citation tracking. Studies were selected using transparent inclusion criteria and underwent quality appraisal using a bespoke checklist based on the AGREE II criteria. Seventeen studies were included in the review, covering five methodologies. A narrative analysis of the selected studies suggests that no single methodology was superior. However, many of the methods shared common components, around which a 'best-practice' framework was formulated. This approach is intended to help inform decision makers' choice of an appropriate risk-ranking study design.

Introduction

Communicable disease outbreaks can pose a significant challenge to public health and to emergency preparedness. Types of threats and the pathogens involved shift in relation to changing factors such as climate change [1,2], global travel and trade [3,4], immigration patterns, urban sprawl, social inequalities [5,6] and other disease drivers [7-10]. An increasingly interconnected world means that diseases emerging in one part of the world, such as Zika, Middle East respiratory syndrome coronavirus or Ebola [11,12] can spread globally. Similarly, diseases once considered tropical can transmit in Europe under the right circumstances [8,13-15].

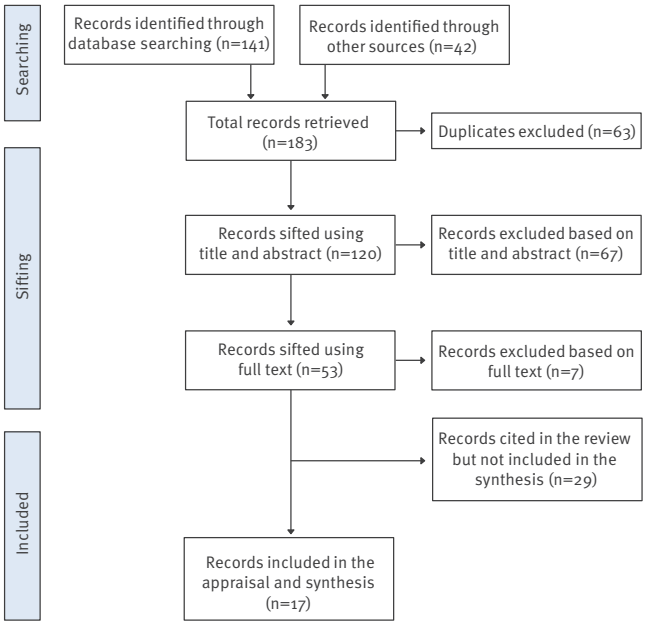
It is essential for public health agencies to be able to account for and assess the rapidly changing global context surrounding communicable disease. One of the Core Capacity Indicators of the International Health Regulations relates to mapping and using priority health risks and resources [16]. This includes conducting national risk assessments for identifying potential 'urgent public health events' as well as the most likely source of these events [16]. At the European level, Article 4 of the European Parliament and Council Decision 1082/2013/EU on serious cross-border threats to health focuses on preparedness and response planning, calling for 'efforts to develop, strengthen and maintain…capacities for the monitoring, early warning and assessment of, and response to, serious cross-border threats to health' [17].

Identifying and prioritising risks are a necessary first phase for informing the public health response to infectious disease risks, and an effective tool to guide strategic planning and ensure the efficient allocation of resources [18]. The need for methodologies to assist national efforts in this area was highlighted at a Joint European Centre for Disease Prevention and Control (ECDC)-World Health Organization (WHO) Consultation on Pandemic and All-Hazard Preparedness, held in Bratislava in November 2013 [19]. Elsewhere, the development of risk-ranking 'toolboxes' has been advocated, which could enable organisations to decide on the best methodologies that are commensurate with ranking exercises [20].

ECDC aims to develop a comprehensive risk-ranking tool for use in strategic prioritisation exercises. There is, however, no current consensus on the best methodology for such risk-ranking exercises, with different organisations proposing different methods. WHO, for example, has produced practical guidance on setting priorities in infectious disease surveillance, advocating a Delphi methodology [21]. Other studies have

Figure 1

Flowchart of search and sifting process, literature review on best practices in ranking communicable disease threats, 2015



varyingly used Delphi, multi-criteria decision analysis (MCDA), the h-index, and a range of other approaches. One commonality is the attempt to guide prioritisation making in situations where evidence is sparse or non-existent.

In order to identify best practices in risk ranking, and to guide further ECDC work in this area, a literature review was initiated to identify and evaluate the range of methods used [22]. The findings from this review were then used to develop a best-practice framework for ranking infectious disease threats.

Methods

The project methodology comprised two key phases. First, a literature review to identify the relevant literature on risk ranking for communicable diseases was conducted. Second, the findings from this review were analysed through a narrative review, which enabled the development of a best-practices framework.

Literature review

The scope of this literature review included all communicable diseases, which are defined according to the European Union (EU) list of communicable diseases for surveillance [23]. For the purposes of this review, risk was defined according to the International Organization for Standardization (ISO) standards with risk being the product of impact and likelihood [24].

Searching

The citation pearl-growing method [25] was used to identify search terms using an initial sample of relevant articles (identified in a scoping search [22]). Searches were performed across biomedical databases (Medline, Embase, Cochrane Library and Centre for Reviews and Dissemination), grey literature (i.e. official documents, non-peer reviewed reports, etc.) and specialist databases (Google Advanced Search, WHO, the World Bank). Subject headings (where available) and variations on search terms related to prioritisation or ranking, were combined with 'communicable' or 'infectious' or 'zoonoses' to search the various sources. Supplemental search techniques of reference harvesting and citation tracking were performed for the initial sample of relevant articles and again for all articles included in the analysis [26].

Sifting

Criteria for inclusion in the review were studies that: described a method of prioritisation/ranking; were published in a peer-reviewed journal or by a national or supra-national government, charity, non-governmental organisation (NGO) or other authoritative institution; were within the geographic scope of the literature review (the EU, Australia, Canada, New Zealand and the United States); were published in English; and were published from January 2000 to December 2014.

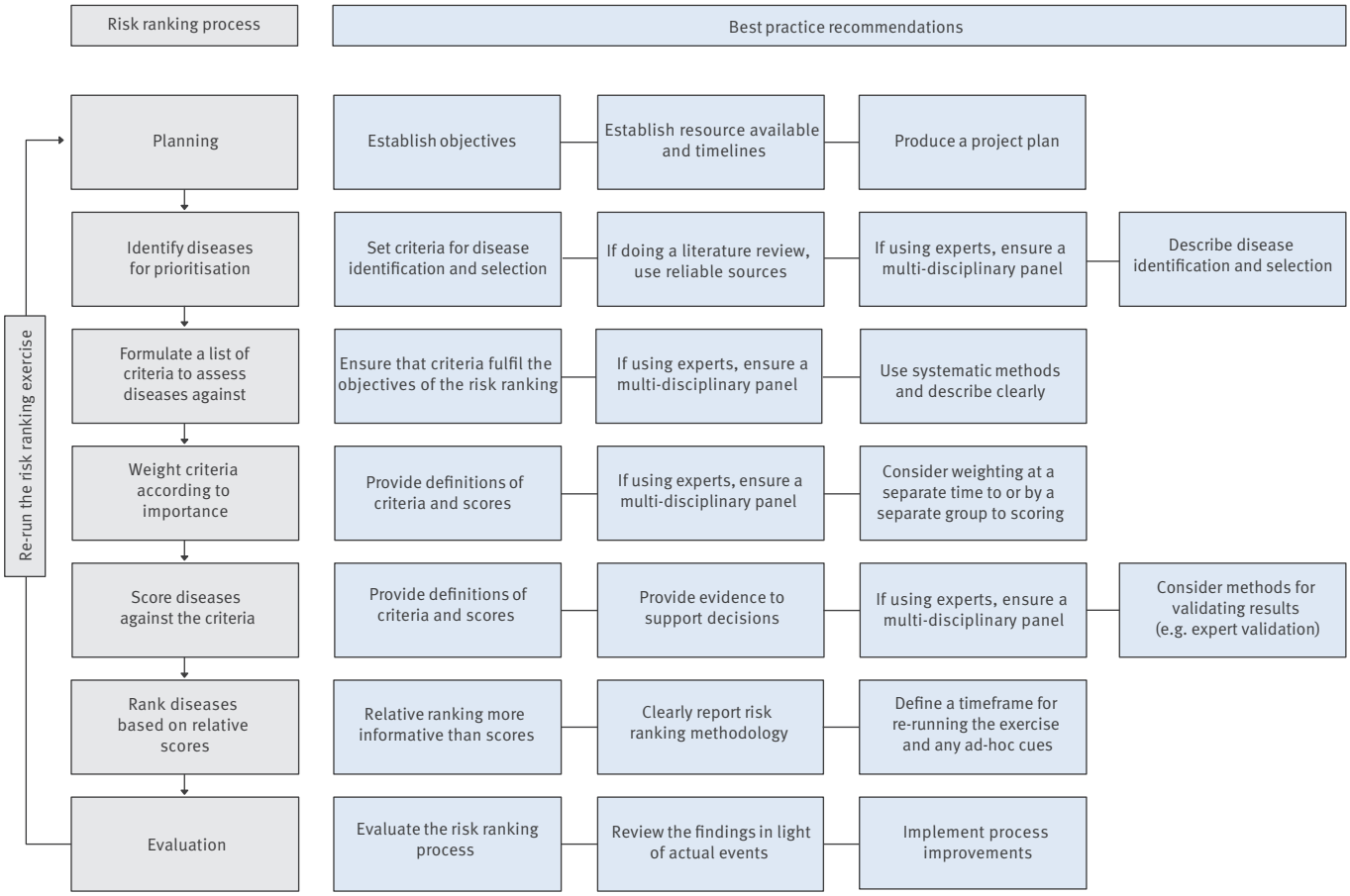
The search and sift process is presented in Figure 1. The searches are not fully exhaustive, although the three-pronged approach is designed to capture the most relevant literature. Studies included in the analysis are presented in Table 1.

Quality appraisal

The aim of the quality appraisal was to evaluate the validity and reliability of individual studies, to enable comparison between individual studies and across different methodologies. No existing checklist was suitable for assessing quality across the different methodologies used in the studies included, and so a quality appraisal checklist was developed [22]. The bespoke appraisal checklist was based on the Appraisal of Guidelines for Research and Evaluation (AGREE) Instrument criteria [27], which evaluates the methodology and reporting of guidelines. The checklist assessed the validity (how well the method measured the important facets of communicable disease) and reliability (internal consistency, inter-rater consistency and precision of the method) of the risk-ranking studies. A sample of quality appraisals was separately appraised by two reviewers to test the checklist and establish rating definitions. Studies were rated according to this set of criteria, and then given an overall rating (Table 2). The qualitative Likert assessments, which are based upon scales that typically range from 'strongly disagree' to 'strongly agree', are represented using a red-amber-green 'traffic light rating system' (with red indicating a high risk of bias likely). Where multiple articles described the same risk-ranking exercise, articles were appraised and extracted as one study, but counted individually within the flowchart (Figure 1) [28-32].

Figure 2

Framework of best practice for risk ranking exercises, for use across methodologies, literature review on best practices in ranking communicable disease threats, 2015



Analysis of best practices in risk ranking

A standardised data-extraction form was used to extract key methodological information. Data extraction was performed in duplicate by two researchers. A narrative synthesis was performed by clustering the studies according to methodology, to compare studies within and across methodologies. The narrative review indicated that no single methodology was superior, but many of the methods shared common components. Therefore a best-practice framework was formulated, structured around the common components identified in the narrative review, which worked across the reviewed methodologies (Figure 2). The best-practice framework is designed to inform decision makers' choice of an appropriate risk-ranking method and ensure that methodologies are carried out according to best practice.

Results

Results from the literature review

Fourteen studies, reported in 17 articles, were selected for inclusion in the review. The studies used one of five methodologies to rank communicable disease risks: bibliometric index [33,34], the Delphi technique [35-38], Multi-Criteria Decision Analysis (MCDA) [31,32,39-41], qualitative algorithms [42,43], and questionnaires [29-31,45 In general, risk-ranking exercises begin with identifying diseases to consider for prioritisation, formulating a list of criteria to assess diseases against, then weighting the criteria according to importance, and scoring diseases against the criteria to create a ranking based on the scores.

Analysis of best practices

Based on the analysis of the studies reviewed, it was possible to comment upon best practice in conducting risk-ranking exercises independent of the methodology selected and based on the steps within this generic process.

This paper focuses on the best-practice framework (Figure 2), which has the overall aim of reducing bias and strengthening the credibility and reproducibility of findings, whichever methodology is used. Some aspects of best practice run across the different steps in the framework, such as using a multidisciplinary team.

Planning

WHO guidance on priority setting in communicable disease surveillance states that planning is an essential step in the process [21]. Establishing the objectives of the exercise enables the selection of an appropriate

Table 1

Characteristics of studies published from January 2000 to December 2014 included in analysis for literature review on best practices in ranking communicable disease threats, 2015

Study	Methodology	Summary
Cox et al. [33]	Bibliometrics (h-index)	651 diseases ranked Primary source: Web of Science Validating source: Pubmed
McIntyre et al. [34]	Bibliometrics (h-index)	1,414 diseases ranked Primary source: Web of Science Validating sources: Google Scholar, Scopus
Balabanova et al. [35]	Delphi study	127 diseases ranked 10 criteria used Criteria weighted 86 participants weighted criteria 20 participants scored diseases 3 point scale used to score diseases 1 round of Delphi scoring
Economopoulou et al. [36]	Delphi study	71 diseases ranked 2 criteria used Criteria not weighted 3 participants scored diseases 56 participants selectively scored diseases 5 point scale used to score diseases 2 rounds of Delphi scoring
Krause et al. [37]	Delphi study	85 diseases ranked 12 criteria used Criteria weighted 11 participants weighted criteria 11 participants scored diseases 3 point scale used to score diseases 1 round of Delphi scoring
WHO et al. [38]	Delphi study	53 diseases ranked 8 criteria used Criteria not weighted 24 participants scored diseases 5 point scale used to score diseases 1 round of Delphi scoring
Cardoen et al. [39]	Multi-criteria decision analysis	51 diseases ranked 5 criteria used Criteria weighted using Las Vegas method 7 participants weighted criteria 35 participants scored diseases Scores of 0–4 points allocated to each disease (based on occurrence and severity)
Cox et al. [31,32]	Multi-criteria decision analysis	9 diseases ranked 40 criteria used Criteria weighted using a qualitative Likert scale (based on likelihood or importance) 64 participants weighted criteria 47 participants scored diseases Likert scale used to score diseases
Havelaar et al. [40]	Multi-criteria decision analysis	86 diseases ranked 7 criteria used Criteria weighted using relative ranking 29 participants Quantitative, scaled values used to score diseases
Humblet et al. [41]	Multi-criteria decision analysis	100 diseases ranked 57 criteria (in 5 categories) 40 participants Criteria weighted using the Las Vegas method Co-efficients of 0–7 points assigned to each option
Morgan et al. [42]	Qualitative algorithm	1 disease ranked (a worked example) 1 participant
Palmer et al. [43]	Qualitative algorithm	5 diseases ranked Number of participants unclear
Horby et al. [44]	Questionnaire studies	61 diseases ranked 5 criteria used Criteria not weighted 518 participants
Ng et al. [28-30]	Questionnaire studies	62 diseases ranked 21 criteria used Criteria weighted using conjoint analysis 4,161 participants

Table 2

Study quality appraisal table for literature review on best practices in ranking communicable disease threats, 2015

Study	Methodology	Overall score	Individual domain scores			Reviewer comments
			Validity	Content validity	Reliability	
Balabanova et al. [35]	Delphi	Amber	Green	Amber	Amber	Sources of bias were identified and mitigated where possible. Implementation issues were not discussed. The criteria used in the study did not meet all of the content validity criteria. Unclear what measures were in place to ensure internal consistency and whether any tests of validity were used.
Cardoen et al. [39]	Semiquantitative methodology (analysed as multi-criteria decision analysis)	Amber	Green	Amber	Amber	Unclear how criteria were developed. Implementation issues were not discussed. Either did not meet or only partly met several of the key communicable disease facets. No measures of internal consistency.
Cox et al. [31,32]	Multi-criteria decision analysis	Green	Amber	Green	Green	Unclear precisely how criteria were developed. Implementation issues were not discussed. Criteria met most of the key communicable disease facets. Sensitivity analyses were used to test validity.
Cox et al. [33]	Bibliometric index	Green	Green	NA	Green	Assessment is based on applicable criteria. This paper did not address any of the key communicable disease facets due to its design. The quality of evidence was not considered. Tested validity by comparing two data sources using Spearman's rank test.
Economopoulou et al. [36]	Delphi	Amber	Green	Amber	Amber	Used two criteria of likelihood and impact. Assessment against content validity domain was based on the facets listed as included in the 'supportive information'; did not include many of those criteria. Implementation issues were not discussed. No measures of internal consistency.
Havelaar et al. [40]	Multi-criteria decision analysis	Green	Green	Amber	Green	Unclear how criteria were chosen. Implementation issues were not fully discussed. Did not meet all of the key communicable disease facets, in particular it did not address mitigation. Participants were sent a repeated exercise to test internal consistency. A sensitivity analysis tested the validity of assumptions made in the different models.
Horby et al. [44]	Questionnaire	Amber	Amber	Amber	Amber	Unclear exactly how criteria were chosen, but they are compared against similar studies. Implementation issues were not discussed. Did not meet all of the key communicable disease facets, across likelihood, impact and mitigation. No tests for internal consistency, although tests to measure variation between professional groups were undertaken.
Humblet et al. [41]	Multi-criteria decision analysis	Amber	Green	Amber	Amber	Addresses some practical issues by stating that their intended methodology was Delphi but they did not have sufficient time. Did not meet all of the key communicable disease facets, but did consider the cost of prevention. No measures of internal consistency, but criteria definitions included to reduce inter-rater variation. Used a probabilistic method to account for variability in scores.
Krause et al. [37]	Delphi	Amber	Amber	Amber	Amber	Implementation issues were not discussed, although practical considerations were included. Did not meet all key communicable disease criteria. Did not measure internal consistency, but results were reviewed by all participants for plausibility. Criteria and scoring definitions were provided to reduce inter-rater variation.
McIntyre et al. [34]	Bibliometric index	Amber	Amber	NA	Amber	Assessment is based on applicable criteria. This paper did not address any of the key communicable disease facets due to its design. The quality of evidence was not considered. Tested validity by comparing two data sources using Spearman's rank test. Authors acknowledge the limitations of the methodology.
Morgan et al. [42]	Qualitative algorithm	Amber	Amber	Amber	Red	It is unclear how this qualitative algorithm was developed, therefore judging the risk of bias was challenging. Implementation issues were not discussed. Questions within the algorithm addressed some of the key communicable disease facets. There were no measures of internal consistency. The algorithm was completed by a single scientist.
Ng et al. [28-30]	Questionnaire	Green	Green	Green	Green	Implementation issues were not specifically discussed, but practical considerations were discussed which would assist implementation. Most of the key communicable disease facets were met. Internal consistency was not measured. The Delphi method reduces the effect of inter-rater variation because of discussion.
Palmer et al. [43]	Qualitative algorithm	Amber	Amber	Amber	Amber	It is unclear how this qualitative algorithm was developed, with most validity criteria partly met or not met. Implementation issues were not discussed. Many key communicable disease criteria were not applicable as this is an early-stage risk assessment. This appeared to be a table-top exercise and it lacked tests of internal consistency and validity.
WHO et al. [38]	Delphi	Amber	Amber	Amber	Amber	Reporting lacked detail, as it was a report of a meeting to give participants experience of such an exercise. Unclear how criteria were developed. Potential sources of bias and mitigations are not reported. The publication was not peer-reviewed and it is unclear if any other review took place. Implementation issues were not discussed but Delphi scoring was limited to one round. Did not meet all of the key communicable disease facets. 95% confidence intervals used to aid discussion of discrepancies in scoring.

Green: criteria met, information related to that item has been clearly reported and all relevant considerations have been made.

Amber: criteria partly met, information related to that item is incomplete, or not all aspects have been considered.

Red: criteria not met, no information provided in the study that is relevant to that item, or information related to that item is very poorly reported.

NA: criteria are not applicable.

Table 3

Scenarios for risk-ranking exercises and suggestions for appropriate methodologies and considerations for their use, literature review on best practices in ranking communicable disease threats, 2015

Scenario	Methodology	Considerations
Rapid or large-scale risk ranking for large number of pathogens	H-index or qualitative algorithm	Both methods are suitable for ranking a large volume of pathogens within a short time period or with limited resources.
Scoping exercise to generate an initial ranking for further study	H-index or qualitative algorithm	As both methods can quickly rank a large volume of pathogens, they can be used to provide a short list for risk ranking using a more comprehensive technique.
Comprehensive risk ranking including novel, emerging and established infections	Multi-criteria decision analysis or Delphi	Both methods provide a comprehensive method for risk ranking. Where resource is restricted, consider limiting the number of criteria or the number of diseases for ranking.
Emerging infections with little published data about them	H-index	In lieu of standard data, such as burden of disease, h-index can indicate a level of professional interest/concern which may be used as an informal proxy measure of disease impact.
	Qualitative algorithm	This method combines expert opinion and evidence (where available). The qualitative nature allows for greater flexibility in decision-making and for the detailed recording of that rationale. This is particularly useful in emerging infections where decisions may be more based on expert opinion than epidemiological data.
	Qualitative algorithm or questionnaires	In qualitative methodologies, including a mechanism for respondents to identify gaps in knowledge or areas for further work could lead to improved evidence upon which to base future decisions.
	Multi-criteria decision analysis	This method can incorporate information from a variety of sources, which is useful in emerging infections where information is sparse. Ranking the risk of alternative scenarios is suitable for situations where there is less certainty about the potential course of the disease. Additionally new information can be incorporated as it emerges, without needing to re-run the entire ranking exercise

methodology that is fit for purpose. All of the methodologies reviewed can be adapted to suit the particular context and requirements of a risk-ranking exercise. Although many of the studies described the objectives of the risk-ranking exercise, they did not provide details of the planning process. Table 3 describes some scenarios in which a risk-ranking exercise might take place, with suggestions for which methodology may be most suited to meet those needs, with a rationale based on the full comparison between and across methodologies from the ECDC technical report [22].

The decision about whether to use qualitative, quantitative or mixed methods should be based on the scope and purpose of the exercise as established during the planning phase. The included studies often provided explanations for their choice of methodology in terms of overcoming or balancing the potential limitations of alternative methodologies, but rarely explained their choice of methods with regards to the specific objectives of their risk-ranking exercise. Five of the reviewed studies used a quantitative methodology [33-35,37,40,41], three used qualitative approaches [36,42,43], and six studies used semiquantitative, mixed methods [28-32,35,38,39,44]. Only four studies used either entirely qualitative or quantitative methods [33,34,42,43], however, these studies were considered by their authors to be most useful as part of a wider risk-ranking exercise rather than as a stand-alone methodology. No comprehensive methodology using only qualitative or quantitative methods was identified in this review.

There are advantages and disadvantages to using quantitative or qualitative methods in different scenarios. For example, in areas where there is little evidence (and what does exist is of poor quality) it may be preferable to use semiquantitative methods (to make best use of the evidence available [39]) or qualitative methods (in recognition that the evidence is not of much help and uncertainty remains [31]). Qualitative data generally takes longer to collect and analyse than quantitative data, although it provides a richness and context to responses that quantitative data cannot. Semiquantitative methods where respondents can provide quantitative scores with qualitative explanations could offer a good balance.

WHO guidance on priority setting in communicable disease surveillance recommends that the planning process includes budgeting, covering all resources required for the ranking exercise [21]. An assessment of the resources required for any of these methods is an important part of the decision-making process. Methods requiring greater resources should not necessarily be disregarded, but the resources required for a risk-ranking exercise affects its feasibility and potentially creates barriers to the study's application by practitioners. Thus, detailed plans should consider resources required at all stages, from the commissioners of the ranking and the deadline for delivery, to the time requirement for each participant in the process

to deliver the ranking. The methods used in any risk-ranking exercise can be adapted to the resource available. For example, where resources are limited the number of criteria can be limited to increase the number of pathogens that can be assessed [37]. There is always the need to balance methodological rigour and real-world practicalities. However, the reliability and validity of the methodology affects the reliability and validity of the output, and therefore whether it will be taken heed of [37].

Identify diseases for prioritisation

Most of the included studies (14 out of 17) described methods used for identifying and selecting diseases for risk ranking. Studies generally used existing surveillance systems to identify diseases and many used notifiable status as one of their selection criteria. Some studies also asked experts to contribute to the list of diseases for ranking, either by suggesting diseases or by commenting on a pre-formulated list. While the reviewed studies reported the method of disease selection, the rationale was generally not detailed and the potential limitations of the method were not explored. For example, using sources such as notifiable disease lists that are based on clinical and laboratory data, combined with suspected risk, would not necessarily be suitable for identifying emerging threats.

Formulate a list of criteria to assess diseases against and weight criteria according to importance

The criteria considered in the studies varied. However, there was a common core of key communicable disease concepts such as how easily the disease could be spread, how reliable diagnostic testing is, the treatability of the disease, impact on school and work absenteeism, and on-going illness resulting from infection. The average number of criteria was 17. The selected criteria should be specific to the context of the exercise (e.g. specific to the purpose of the exercise, the country where it is taking place): for example some studies considered the role of public concern/perception whereas many did not. Preventive measures currently in place (e.g. vaccinations) should also be considered as criteria so that diseases with low incidence due to effective control measures are not deprioritised and risk resources being allocated elsewhere [35]. The studies that weighted criteria according to importance did so using expert opinion, which creates potential subjectivity and inconsistency in weightings. Including clear definitions of criteria can help to reduce this potential bias [37,41]. Weighting can be assigned to criteria using different methods such as the Las Vegas method [45], allocating differing numbers of points to criteria, or simple relative ranking.

One study engaged members of the public, but they were included only in the initial focus groups to identify and weight criteria [28-30].

Score diseases against the criteria

Most studies scored diseases based on expert opinion, except for the qualitative algorithms [42,44], which provided a relative ranking, and the studies using h-index scores to rank diseases [33,34]. The incorporation of expert opinion in 13 out of 17 studies suggests that it provides a unique input that would be otherwise missing from risk-ranking exercises. The average number of experts included was 231; however, there were some outliers and so the median value of 59 (interquartile ratio: 45) may be a more useful indication. None of the included studies described how they assessed whether sufficient numbers of participants were included, and therefore it would be helpful if future studies indicated how their sample sizes were determined. Most of the studies reported how their participants were selected, which provides useful information for those seeking to apply these methods to their own setting. Multidisciplinary input based on expertise and experience can help to inform decisions where standard data are not available, such as in the case of emerging disease threats or areas with great evidential uncertainty. The variability and subjectivity of scoring decisions between individuals and between different professional groups is a potential source of bias in risk ranking. While expert input introduces potential bias, it is needed where clear quantitative metrics are not available or where they are not easily comparable. Measures can be put in place to mitigate these risks, such as clear explanations of criteria and definitions of scores to reduce inter-rater variation and interdisciplinary discussion of scores [35,37]. Formal statistical methods, such as Kappa scores, can be used to measure variation between individuals and professional groups, and appropriate adjustments can be made if the variation is considered too high. Alternatively, allowing participants to qualitatively explain their scores could be useful to assess potential causes of variation. Incorporating a method whereby participants can express uncertainty in their scoring can help to understand the rationale behind responses, identify where expert opinion disagrees with current evidence or identify areas for further research [39,44]. When incorporating expert opinion into any methodology, it is necessary to consider the representativeness of the people whose opinion is sought and, as the reviewed studies did, engage a range of multidisciplinary specialists to cover the different aspects of communicable disease risk ranking. There can be conflict between the desire to engage a variety of participants and the need to ensure that those participants are making informed decisions. This risk can be mitigated, for example by allowing respondents to acknowledge the limits of their knowledge [39], or using qualitative scales or visual representations to aid participants in interpreting otherwise abstract scores [31,32].

Five studies provided participants with evidence to support their decision-making. This evidence was collated from reliable sources such as national governments, supranational organisations (such as the EU), NGOs

(such as WHO), and charities. Providing such evidence could be interpreted as prejudicing the impartiality of the decision-making by providing information to help steer responses. However, providing evidence may help to reduce subjectivity, reduce bias (individual or professional), correct misconceptions and ensure that participants are making decisions based on reliable, up-to-date information that is relevant to the purpose of the exercise. All tools, regardless of methodology, are reliant on the quality and availability of evidence upon which to base judgments. Morgan incorporated references of the evidence used in decision-making into their qualitative algorithm [42], so that the basis of the decision could be understood and scrutinised. Decision-making should record the evidence upon which it is based, the quality of that evidence and whether any evidence gaps exist.

Rank diseases based on relative scores

Some studies reported that an indication of overall trend [44] or relative ranking was more informative than the raw individual scores of pathogens [37,40]. Various mathematical techniques were used to combine scores, depending on the methodology. As with other steps in the process it is necessary to clearly communicate the process from scoring pathogens against weighted criteria to ensure transparency and reproducibility of the method.

Evaluation

The studies included did not provide information on an evaluation of the effectiveness of the process and its output. Krause stated that the current exercise was based on experience of a previous exercise and would be further refined in future [37]. WHO guidance emphasises the role of risk-ranking exercises in the evaluation of surveillance measures and places it within a process cycle, which includes evaluation [21]. Evaluation is included in the best-practice framework, despite not being explicitly included in the reviewed studies, because it is recommended in WHO guidance [21] and is generally considered central to implementing and improving new processes. Using a process improvement cycle such as 'Plan, Do, Study, Act' (PDSA) [46] provides a framework for evaluating the process, comparing the rankings with actual events and enabling process improvements that can be implemented when the exercise is repeated.

Re-run the risk-ranking exercise

Placing risk-ranking exercises within a process-improvement cycle such as PDSA [46] assists in the evaluation of the process and its outcomes, but also emphasises the need to repeat the risk-ranking exercise. Krause et al. state that the experience of the current risk-ranking exercise will inform future exercises [37]. However, none of the studies lay out specific timescales or triggers for the risk-ranking exercise to be repeated. As such it is not possible to derive specific best practice in this area. However, as part of a cycle of activities, risk-ranking exercises should be re-run periodically (every five years), depending on an assessment of the extent to which the various disease drivers have changed. It is also necessary to consider triggers – such as evidence of emerging threats, the development of new interventions or new surveillance intelligence for current threats [42] – that could cue a re-ranking of diseases. In such cases it may be possible to perform an interim and rapid assessment before the next scheduled risk-ranking exercise is due.

Resource requirements

Not all studies reviewed here included information about the time and human and financial resources involved in the risk-ranking exercise. Such practical information would inform the choice of methodology and also any pragmatic modifications (such as reducing the number of pathogens included) that might be made to make the exercise viable. Some studies alluded to their method being time-consuming [35,37] or that time constraints required them to adapt their methodology or switch to another method [28-30,38,41]. General discussion of how methods can be adapted to suit time or resource constraints were discussed in some papers [31,32,36,37,40], such as reducing the number of diseases considered to allow for a larger Delphi panel [37]. One study provided data on how long the survey, which was one part of the exercise, took for participants to complete (27 min in Canada and 28 min in the US) [28-30]. In addition to the time of staff and participants, resources such as specialist software [28-30], staff training (e.g. in software or statistical methods) or outside costs (e.g. using a firm to recruit participants, hiring external skills such as focus group facilitators) were not reported.

Discussion

Predicting the future risk of communicable diseases is challenging as there are many changing factors and unknowns [1,7,18,47]. This literature review aimed to identify and evaluate the range of methods available for risk ranking of communicable diseases. The study characteristics are summarised in Table 1 and quality appraisal results are provided in Table 2. Given the diversity of methods available, it was not possible to recommend a single methodology for use in risk-ranking exercises. This finding was echoed by a scan of systematic reviews of risk ranking in other sectors including biological agents [20], pathogens, pests and weeds [48], and bioterrorism agents [49].

A best-practice framework was therefore developed using a process based on the common components identified in the studies included our literature review. It is an adaptable framework that can be applied to a variety of specific methodologies and provides best-practice recommendations to promote best practice across the various methodologies identified. We validated it by cross-checking it against the common themes of good practice identified in a systematic review of health research priority setting [50], and a

conceptual framework with recommendations for successful health service priority setting [51].

It is noteworthy that periodic evaluations of risk ranking were not explicitly considered in many of the studies reviewed here. Ultimately, risk ranking is best viewed as an initial part of the process of strategic public health planning, with the key objective being strengthened strategies to mitigate communicable disease spread. Given the rapidly changing public health landscape, it is advisable to repeat risk-ranking exercises at regular intervals. In addition, as has been observed elsewhere, there is value in the risk-ranking process itself, which has the potential to bring together stakeholders and practitioners from diverse fields to promote interdisciplinary working [52].

Limitations

This review focused only on ranking exercises conducted for communicable diseases. Methodologies from other sectors might also be relevant, but were not considered here. A limitation of the review is that the search, sift, quality appraisal and analysis was undertaken by a single researcher. However, quality assurance measures were put in place to mitigate any potential bias. The search strategy and approach were peer reviewed. Sifting decisions were made according to pre-defined criteria to ensure consistent decision-making. A sample of quality appraisals were duplicated to inform the development and refinement of the quality appraisal checklist, and establish scoring definitions to ensure consistent ratings. Data extractions were duplicated to ensure consistency and to check that the table captured the information required for analysis. The use of a single quality appraisal checklist across different methodologies means that the appraisal was not as deep as if method-specific appraisal tools had been used. However, the use of a single appraisal checklist enabled comparisons to be made across studies based on the principles of validity and reliability, regardless of the precise methodology. As with all quality appraisals based on published reports, the quality appraisal was affected by the reporting quality. Therefore criteria being 'not met' means that this detail was not reported in the study, however, there may be some discrepancy between the actual methodology and what was reported. Although most of the studies included in this review reported their findings clearly, there were some instances where there were gaps in reporting, which affected quality appraisals and analysis. Clear reporting ensures that processes are transparent, a stated aim of most of the included studies, so that the process can be understood and assessed by multiple stakeholders. Furthermore, it enables others to replicate, develop and improve upon previous practice, leading to improvements in methodologies.

Conclusions

The methodologies identified in this review mostly followed common approaches to risk ranking. The choice of methodology should reflect the purpose of the risk-ranking exercise. Common best-practice approaches, such as engaging diverse panels of stakeholders, and clearly delineating ranking criteria and criteria weights, were identified. The insights from this study will inform subsequent ECDC work on risk ranking, and should be relevant to any audience interested in ranking risks.

Conflict of interest

None declared

Authors' contributions

EOB: literature searches; study selection; writing. EOB and RT: generated study design; designed and performed quality appraisals; data extractions; editorial contribution. KG: expert input into study design and methodology; editorial contribution. JES and MC: initiating the study, study design input; editorial contribution. All authors have reviewed and approved the final article.

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World Health Organization announces European Region malaria free

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On 20 April 2016, the World Health Organization (WHO) announced that the WHO European Region, which comprises 53 countries, is the first of the WHO regions to have interrupted the indigenous transmission of malaria [1].

In 2005, the WHO European Regional Office for Europe adopted the Tashkent Declaration, 'The Move from Malaria Control to Elimination' [2] which paved the way for a new malaria elimination strategy, the 'Regional Strategy: From Malaria Control to Elimination in the WHO European Region 2006-2015' [3]. The Regional Strategy set out milestones for the countries of the WHO European Region to eliminate malaria. Between 1995 and 2015, the number of indigenous malaria cases went from around 90,000 to zero in the European Region.

In July 2016, the WHO will hold its first meeting on the prevention of the re-introduction of malaria into the WHO European Region. According to the WHO, the meeting will focus on prevention through (i) sustained political commitment, (ii) strong vigilance to test and treat all malaria cases promptly, (iii) understanding how malaria transmission could be reintroduced and the risk it poses; and (iv) immediate action if local malaria transmission resumes.

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