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Indistinguishable NDM-producing *Escherichia coli* isolated from recreational waters, sewage, and a clinical specimen in Ireland, 2016 to 2017

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In this study, New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacteriaceae* were identified in Irish recreational waters and sewage. Indistinguishable NDM-producing *Escherichia coli* by pulsed-field gel electrophoresis were isolated from sewage, a fresh water stream and a human source. NDM-producing *Klebsiella pneumoniae* isolated from sewage and seawater in the same area were closely related to each other and to a human isolate. This raises concerns regarding the potential for sewage discharges to contribute to the spread of carbapenemase-producing *Enterobacteriaceae*.

We report the finding of New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacteriaceae* in fresh water and seawater samples collected at two beaches located near an untreated human sewage ocean discharge. Isolates of NDM-producing *Escherichia coli* derived from the sewage collection system, the sewage storage tank and the outflow were 100% identical by pulsed-field gel electrophoresis (PFGE) to those derived from a fresh water stream on one of the beaches, and to a clinical isolate.

Recreational water and sewage sample sites

In 2016, we identified a beach (Beach A) in Ireland, used for bathing and recreation, which is crossed by two fresh water streams (Stream A and Stream B), flowing from the surrounding countryside. These streams were examined for the presence of carbapenemase-producing *Enterobacteriaceae* (CPE). The detection of NDM-producing *E. coli* in these waters prompted subsequent additional sampling of the streams. As untreated human sewage was being discharged into the sea in the vicinity of the beach, and the fresh water streams can become immersed in seawater at high tide, sewage was evaluated as a potential source. Sewage samples included samples from the collection system, the storage tank and the outflow. Sampling was performed in the period May to September, 2016. The sewage system is not linked to any hospital or long-term care facility that we are aware of. Further sampling of the fresh water streams and sewage sites was carried out in January 2017. In addition to this, seawater from Beach A and from a second beach (Beach B), ca 950 m in a direct line from Beach A were examined. Figure 1 shows a schematic diagram of the sampling points and their location relative to each other.

Processing of samples

We applied a previously described method (CapE), to examine large volumes of water (30L) from both the fresh water streams and the seawater, for the presence of CPE [1]. Following filtration and overnight enrichment, the samples were sub-cultured onto Brilliance CRE agar (Oxoid). Sewage samples were examined by direct plating onto Brilliance CRE agar. Following purification, presumptive isolates were identified to species level by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry, and antimicrobial susceptibility testing was performed and interpreted in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [2]. Carbapenemase-encoding genes were detected by real-time PCR, as previously described [3-6]. Typing of NDM-producing *Enterobacteriaceae* was not performed at the variant level, but PFGE was performed on all isolates, as outlined previously [7]. PFGE profiles of NDM-producing *Enterobacteriaceae* isolated from recreational water and sewage samples were compared with

Figure 1

Schematic diagram of water and sewage sampling points and their location relative to each other, Ireland, 2016–2017

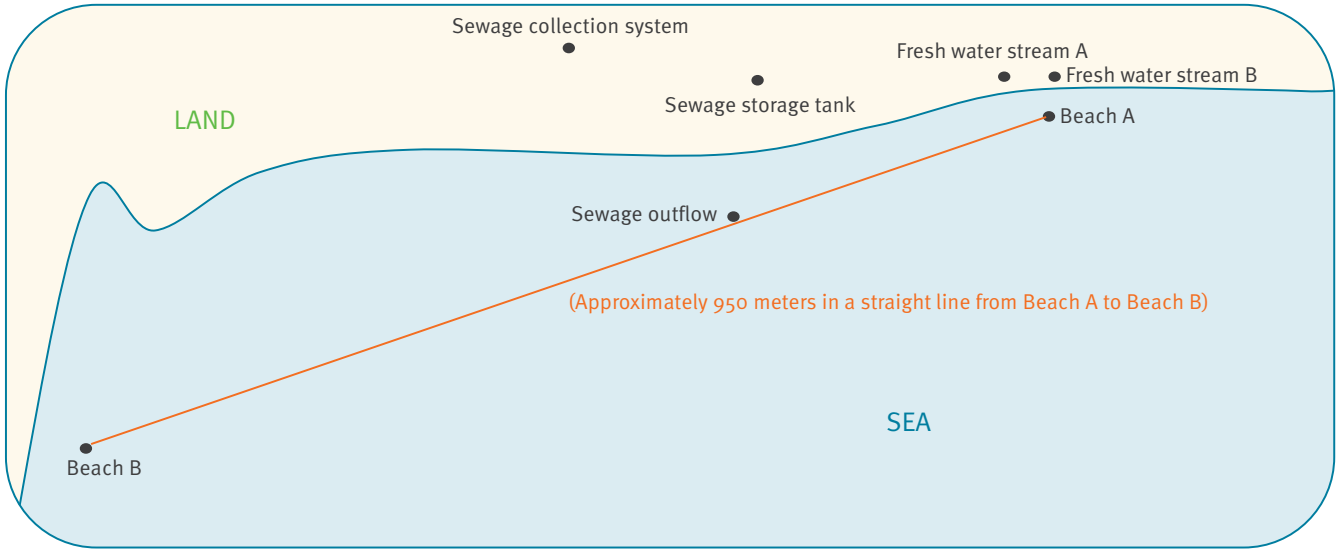
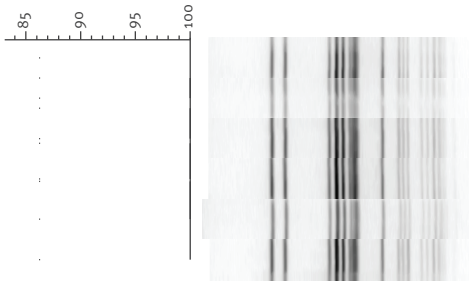


Figure 2

PFGE analysis of New Delhi metallo-beta-lactamase-producing *Escherichia coli* isolated from fresh water, sewage and a clinical source in Ireland, 2016–2017 (n = 6 isolates)



Isolate number	Bacterium	Source of isolate	Date sample was taken
418	*E. coli*	Fresh water stream B	13 Jul 2016
523	*E. coli*	Fresh water stream B	24 Aug 2016
567	*E. coli*	Sewage storage tank	15 Sep 2016
574	*E. coli*	Sewage outflow	15 Sep 2016
581	*E. coli*	Sewage collection system	15 Sep 2016
C2	*E. coli*	Clinical specimen	Feb 2016

PFGE: pulsed-field gel electrophoresis.

PFGE typing was performed with *Xba1*. All isolates are 100% similar by PFGE.

PFGE profiles of NDM-producing *Enterobacteriaceae* isolated from clinical specimens.

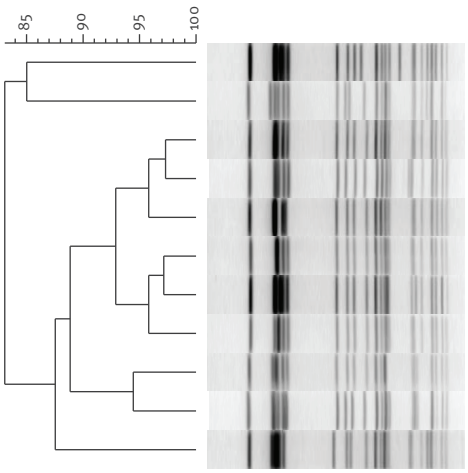
Findings of New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacteriaceae*

Of eight fresh water samples from Stream B, NDM-producing *E. coli* were isolated from two samples, which were collected on 13 July and 24 August 2016. NDM-producing *E. coli* were also isolated in samples collected on 15 September 2016 from the sewage collection system (one of two samples), the storage tank (one of two samples) and the sewage outflow (one of one sample) (Table). All isolates were resistant to ampicillin, cefotaxime, cefoxitin, cefpodoxime, ceftazidime, ciprofloxacin, ertapenem, meropenem and nalidixic acid. The isolates obtained from the fresh water and sewage samples are indistinguishable by PFGE analysis from a human isolate submitted to the National Carbapenemase Producing *Enterobacteriaceae* Reference Laboratory Service (CPERLS) in early 2016 (Figure 2).

NDM-producing *K. pneumoniae* were isolated from two of three sewage sampling sites on 15 September 2016 and two of two sewage sampling sites on 18 January 2017. NDM-producing *K. pneumoniae* was also detected in seawater samples collected at Beach A and Beach B

Figure 3

PFGE analysis of New Delhi metallo-beta-lactamase-producing *Klebsiella pneumoniae* isolated from seawater, sewage and a clinical source in Ireland, 2016–2017 (n = 11 isolates)



Isolate number	Bacterium	Source of isolate	Date sample was taken
626	*K. pneumoniae*	Sewage collection system	18 Jan 2017
628b	*K. pneumoniae*	Seawater Beach B	18 Jan 2017
625	*K. pneumoniae*	Sewage storage tank	18 Jan 2017
628c	*K. pneumoniae*	Seawater Beach B	18 Jan 2017
621c	*K. pneumoniae*	Seawater Beach A	18 Jan 2017
568	*K. pneumoniae*	Sewage storage tank	15 Sep 2016
582	*K. pneumoniae*	Sewage collection system	15 Sep 2016
C3	*K. pneumoniae*	Clinical specimen	Feb 2016
621b	*K. pneumoniae*	Seawater Beach A	18 Jan 2017
628d	*K. pneumoniae*	Seawater Beach B	18 Jan 2017
621d	*K. pneumoniae*	Seawater Beach A	18 Jan 2017

PFGE: pulsed-field gel electrophoresis.

PFGE typing was performed with *Xba1*. Isolates are between 83% and 97% similar by PFGE

on 18 January 2017 (Table). These isolates were resistant to ampicillin, cefotaxime, cefoxitin, cefpodoxime, ceftazidime, ciprofloxacin, ertapenem, gentamicin, kanamycin, meropenem, nalidixic acid and tetracycline. PFGE analyses of isolates from sewage, seawater and a human isolate (from CPERLS) show isolates to be between 83% and 97% similar (Figure 3).

Discussion

The rapid dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE) in Europe and worldwide is making the delivery of effective healthcare an increasing challenge [8]. The number of CPE confirmed by the national reference laboratory in humans in Ireland has increased every year, rising from 48 in 2013, to 369 in 2016. In 2016, the three most commonly reported carbapenemases in Ireland were *K. pneumoniae* carbapenemase (KPC), carbapenem-hydrolysing oxacillinase-48 (OXA-48), and NDM [9]. The NDM gene is primarily plasmid-encoded, enabling its easy transfer between bacterial species. The plasmids are diverse and usually harbour a large number of other resistance genes [10]. The NDM gene has been detected extensively in the Indian subcontinent where it has been reported from both environmental and clinical sources [11]. Rapid global spread has been aided greatly by intercontinental travel [12]. However, a recent study in 2016 reported an outbreak of NDM-1-producing *Enterobacteriaceae* in a number of hospitals in Ireland, where links to foreign travel were not identified [13].

In Europe, a number of studies have reported the presence of CPE in recreational water, including Verona integron-encoded metallo-beta-lactamase (VIM) producing *K. pneumoniae* in a river in Switzerland in 2013 [14], KPC-producing *E. coli* in a river in Portugal in 2012 [15], VIM-1, VIM-34, and IMP-type metallo-beta-lactamase (IMP)-8 producing *E. coli* in the same Portuguese river in 2016 [16], and NDM-1 producing *K. pneumoniae* in the River Danube in Serbia, in 2016 [17]. Here we identify NDM-producing *Enterobacteriaceae* in environmental water samples collected at two adjacent beach sites in Ireland. As far as we are aware, this is the first such finding in bathing seawater in Europe.

We consider that contamination of the environment with NDM-producing *Enterobacteriaceae* from the human sewage outflow is likely to be the source, and that the fresh water streams were contaminated by backwash of sewage onto the beach by tidal currents. The presence of NDM-producing *Enterobacteriaceae* in the bathing water (seawater) and at a separate bathing site ca 950 m in a direct line indicates the extent of this contamination. It is important to note that by the established regulatory standards, the bathing water quality in the area concerned has been consistently of sufficient quality [18]. Notwithstanding compliance with regulatory standards however, it is reasonable to conclude that those using a beach such as this for recreational purposes might be at least intermittently

Table

Overview of sampling sites, dates and detection of carbapenemase-producing *Enterobacteriaceae* in a coastal region in Ireland, 2016–2017

Sample site	Date of sampling	Carbapenemase-producing *Enterobacteriaceae*
Fresh water Stream A	25 May 2016	Not detected
	22 Jun 2016	Not detected
	13 Jul 2016	Not detected
	10 Aug 2016	Not detected
	24 Aug 2016	Not detected
	7 Sep 2016	Not detected
	15 Sep 2016	Not detected
	18 Jan 2017	Not detected
Fresh water Stream B	25 May 2016	Not detected
	22 Jun 2016	Not detected
	13 Jul 2016	NDM-producing *E. coli*
	10 Aug 2016	Not detected
	24 Aug 2016	NDM-producing *E. coli*
	7 Sep 2016	Not detected
	15 Sep 2016	Not detected
	18 Jan 2017	Not detected
Sewage storage tank	15 Sep 2016	NDM-producing *E. coli*
		NDM-producing *K. pneumoniae*
	18 Jan 2017	NDM-producing *K. pneumoniae*
Sewage collection system	15 Sep 2016	NDM-producing *E. coli*
		NDM-producing *K. pneumoniae*
	18 Jan 2017	NDM-producing *K. pneumoniae*
Sewage outflow	15 Sep 2016	NDM-producing *E. coli*
Seawater Beach A	18 Jan 2017	NDM-producing *K. pneumoniae*
Seawater Beach B	18 Jan 2017	NDM-producing *K. pneumoniae*

E. coli: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*; NDM: New Delhi metallo-beta-lactamase.

exposed to NDM-producing *Enterobacteriaceae*. Although, to date, there is no evidence that NDM-producing *Enterobacteriaceae* has been acquired as a result of exposure to this beach environment, Leonard et al. have recently reported on the level of risk of exposure to antibiotic resistant bacteria in coastal waters and its relationship to different types of water sports [19].

It appears therefore that there is potential for environmental contamination to contribute to a transition of CPE from largely healthcare-associated organisms, to organisms affecting the general population and the veterinary sector. From a public health perspective, the findings focus attention on the need to accelerate programmes to cease discharge of untreated sewage into the environment. This practice should be unacceptable in the context of discharges in the vicinity of popular bathing and recreation areas where human exposure is highly likely.

We consider that our findings point to potential limitations of the use of *E. coli* as an indicator bacteria for bathing water quality based on the number of colony forming units (CFU) per 100 mL [20]. In our view, this approach does not reflect the pathogenicity of some variants of *E.* coli, such as Shiga-toxigenic *E. coli* for which the infectious dose is very low, (<10 CFU/mL) [21].

Conflict of interest

None declared.

Authors' contributions

Sampling was carried out by Bláthnaid Mahon, Carina Brehony, Dearbháile Morris, Paul Hickey and Shane Keane. Ann Dolan facilitated gaining access to the sewage supply. Water samples were filtered by Carina Brehony, James Killeen and Bláthnaid Mahon. Screening of samples for CPE was carried out by Bláthnaid Mahon. MALDI-TOF, antimicrobial susceptibility testing and PFGE was completed out by Bláthnaid Mahon, and real-time PCR by Elaine McGrath. Elaine McGrath and Belinda Hanahoe provided access to the clinical isolates. Bláthnaid Mahon, Dearbháile Morris and Martin Cormican were responsible for the study design and coordination, and wrote the manuscript. All authors were involved in reviewing the manuscript and approved the final version.

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Emergence of new recombinant noroviruses GII.p16-GII.4 and GII.p16-GII.2, France, winter 2016 to 2017

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An early increase in outbreaks of norovirus gastroenteritis characterised at the French National Reference Centre occurred this winter season. They were concurrent with an unusual pattern of circulating strains, with three predominant genotypes: the re-emergent variant GII.P4 2009-GII.4 2012 found in 28% of norovirus outbreaks and two new emergent recombinant strains GII.P16-GII.4 2012 and GII.P16-GII.2 never before observed in France, found in 24% and 14% of norovirus outbreaks, respectively.

We report an early increase in norovirus (NoV) gastroenteritis outbreaks investigated during this 2016/17 season at the French National Reference Centre for Gastroenteritis Viruses (NRCgev), compared with the previous season (Figure 1). Molecular characterisation and phylogenetic analysis of the strains responsible for these outbreaks showed that three predominant genotypes were co-circulating, including two new emergent recombinant strains never before observed in France.

Laboratory investigation

From week 40 in 2016 to week 3 in 2017, 350 stool samples corresponding to 114 gastroenteritis outbreaks were investigated at the French NRCgev. NoV detection was performed by real-time RT-PCR as previously described [1]. A total of 222 stool samples, corresponding to 87 outbreaks (76%), were positive for norovirus. In comparison, during the same period in 2015/16, 55 of 76 outbreaks (72%) had been positive for norovirus (Figure 1). Interestingly, the increase in norovirus-positive outbreaks started earlier this winter season than in the previous season.

Two to three norovirus-positive specimens from each positive outbreak were genotyped as previously described [1], by sequencing a fragment of the RNA polymerase gene (open reading frame (ORF) 1) and a fragment of the capsid gene (ORF2). Genotype was determined using the Norovirus Genotyping Tool version 1.0 [2]. Furthermore, for a selection of samples for which ORF1 and ORF2 presented different genotypes, direct sequencing of a 1,112 bp region spanning the 3' end of ORF1 and the 5' end of ORF2 was performed to confirm the recombination status. Amplification was performed using the primer set JV12/G2SKR. ORF1-ORF2 amplification and sequencing confirmed a recombination event for 27 samples. Nucleotide sequences of these samples were submitted to the GenBank database under accession numbers KY817495 to KY817521. Figure 2 presents the diversity of NoV genotypes found in the current and the previous season, between week 40 and week 3.

Three genotypes were predominant this season: the variant GII.P4 2009-GII.4 2012 found in 24 of 87 norovirus outbreaks (28%), the recombinant GII.P16-GII.4 2012 in 21 outbreaks (24%), and the recombinant GII.P16-GII.2 in 12 outbreaks (14%). Furthermore, 12 strains could only be partially characterised, 10 with a GII.4 2012 capsid and two with a GII.2 capsid. In comparison, one single genotype GII.P17-GII.17 had predominated during the 2015/16 season (54% of outbreaks), a genotype that was rarely found at the beginning of the current season (n = 5; 6%).

Phylogenetic analysis showed that all the GII.P4 2009-GII.4 2012 strains found in this study clustered with the strain GII.P4 2009-GII.4 2012 (GenBank KF199164) found in Denmark in March 2013 [3], in both the polymerase and capsid regions (Figures 3 and 4).

They also clustered in the sequenced capsid fragment with the reference strain GII.4 Sydney 2012 (JX459908) and with the GII.4 Melbourne 6623 (KX767083) found in Australia in June 2016 [4]. The polymerase region of the GII.P16-GII.4 2012 strains and GII.P16-GII.2 strains were all closely related to the GII.P16-GII.16 strain VannesL23/1999/FR (AY682551), but interestingly, they separated in two distinct clusters (Figure 3). Of note,

Figure 1

Cumulative number of norovirus outbreaks investigated at the French National Reference Centre for Gastroenteritis Viruses, France, week 40 to week 3, 2015/16 (n = 55) and 2016/17 (n = 87)

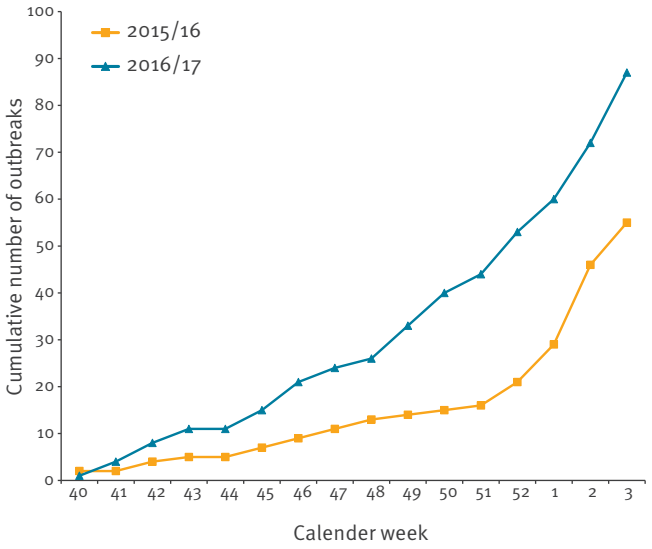
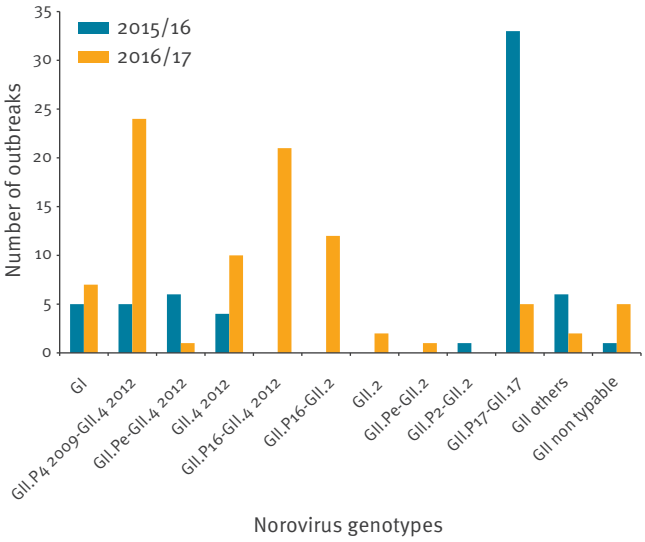


Figure 2

Diversity of norovirus genotypes found at the French National Reference Centre for Gastroenteritis Viruses, France, week 40 to week 3, 2015/16 (n = 61) and 2016/17 (n = 90)



the polymerase sequence of the new recombinant GII.P16-GII.2 GO831GerNRW (KY357449) found in Germany this winter season [5] appeared in the same cluster as the French GII.P16-GII.2 strains.

In the capsid region, the GII.P16-GII.2 strains clustered with the reference strain Melksham/1994/UK (X81879) and were closely related to the recombinant GII.P16-GII.2 GO831GerNRW (KY357449) (Figure 4). Of note, two GII.2 capsid sequences for which no polymerase sequence could be determined also appeared in the same cluster. The capsid sequences of the GII.P16-GII.4 2012 strains were closely related to the reference strains GII.4 Sydney 2012, but interestingly, they segregated in a clearly distinct cluster from the GII.P4 2009-GII.4 2012 (Figure 4). It has to be noted that of the 10 GII.4 2012 strains for which polymerase gene amplification and sequencing failed, eight clustered with the GII.P16-GII.4 2012 strains and two clustered with the GII.P4 2009-GII.4 2012 strains, suggesting that the former may bear a GII.16 polymerase genotype while the latter may bear a GII.4 polymerase genotype.

Discussion

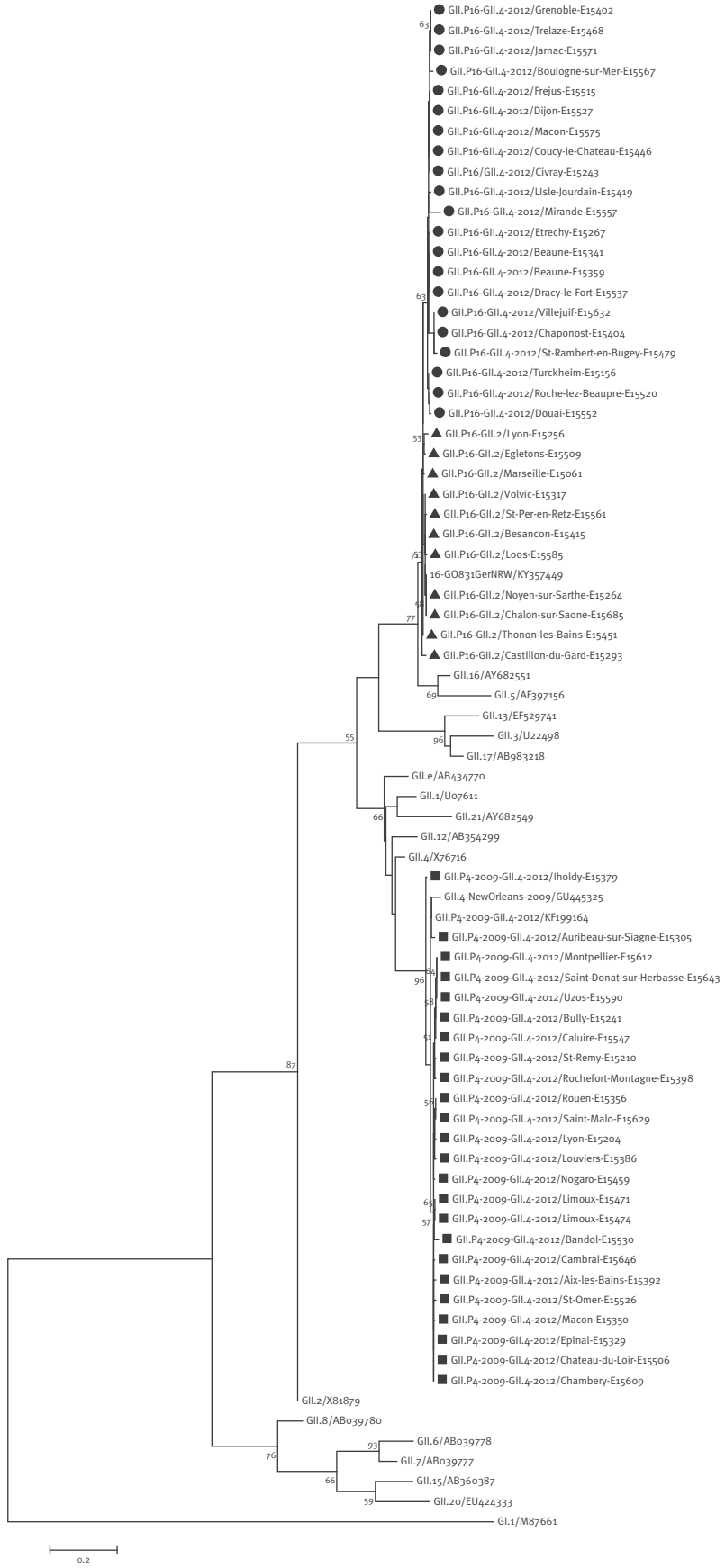
We observed an unusual co-circulation of three norovirus strains this winter season, including two emergent recombinant strains never before detected in France. The co-circulation of two strains has occasionally been observed, such as the 2006a with the 2006b variant, but this was geographically and temporally limited [6]. Usually and for more than 20 years, gastroenteritis epidemics reported all over the world have been linked to a single predominant strain, principally a succession every two to three years of GII.4 genotypic variants, including US95/96 1996, Farmington Hills 2002, Hunter 2004, Den Haag 2006b, New Orleans 2009 and Sydney 2012 [6-8]. Unexpectedly, in the winter of 2014/15, a GII.17 strain emerged in Asia and then replaced the previously predominant GII.4 Sydney 2012 [9]. In France, the GII.17 strain became predominant in the winter of 2015/16 (data not shown).

One of this season's predominant strains, the variant GII.P4 2009-GII.4 2012, had already been detected in France during the seasons 2012/13, 2013/14 and 2014/15, at a time when the variant Sydney 2012 largely predominated, and to a lesser extent in 2015/16, when the strain GII.17 predominated. This variant was described in Denmark and Italy during the season 2012/13 [3,10] and more recently in Australia in August 2015 and as an altered version in June 2016 [4]. Interestingly, the Australian authors suggested that this current recombinant strain could have the potential to become a pandemic variant [4]. However, the partial sequences of the capsid gene obtained in our laboratory do not provide enough information to differentiate between the 2012/13 variant and the derivative, and further molecular investigations are needed.

The two recombinant strains GII.P16-GII.4 2012 and GII.P16-GII.2 had never been observed in France before this winter season and have to our knowledge never been reported as major strains responsible for outbreaks in any country before this season. Although they were circulating concurrently this season in Germany, the reported pattern of circulating strains was different from what was observed in France [5]. Indeed, the GII.P16-GII.2 was the predominant strain responsible for 42% of outbreaks in Germany, far ahead of the variant GII.P4 2009-GII.4 2012 (10%) and the recombinant GII.P16-GII.4 2012 (10%), while in France it was third after GII.P4 2009-GII.4 2012 and GII.P16-GII.4. The reasons

Figure 3

Phylogenetic tree based on the partial nucleotide sequences (287 bp) of the norovirus polymerase gene



Phylogeny was reconstructed using the maximum-likelihood method implemented in MEGA6 [11] with the Kimura 2-parameter substitution model (i.e. best nucleotide substitution model for the dataset). The number of substitutions per site is indicated by the scale bar. Bootstrap values were calculated for 500 replicates and are indicated at each node when ≥ 50%. French norovirus strains from this study are labelled as follows: full circles for GII.P16-GII.4, full triangles for GII.P16-GII.2 and full squares for GII.P4-GII.4.

Figure 4

Phylogenetic tree based on the partial nucleotide sequences (266 bp) of the norovirus capsid gene

Phylogeny was reconstructed using the maximum-likelihood method implemented in MEGA6 [11] with the Kimura 2-parameter substitution model (i.e. best nucleotide substitution model for the dataset). The number of substitutions per site is indicated by the scale bar. Bootstrap values were calculated for 500 replicates and are indicated at each node when≥50%. French norovirus strains from this study are labelled as follows: full and empty circles, respectively, for GII.P16-GII.4 and GII.4 strains probably bearing a GII.P16 genotype; full and empty triangles, respectively, for GII.P16-GII.2 and GII.2 strains probably bearing a GII.P16 genotype; full and empty squares, respectively, for GII.P4-GII.4 and GII.4 strains probably bearing a GII.P4 genotype.

for these differences are unclear. One could be the setting of the outbreaks, since the majority of the investigated outbreaks in France occurred in nursing homes (73%), while in Germany, they occurred mainly in childcare facilities (56% vs 17% in nursing homes). However, a complete assessment will be necessary at the end of the season to draw any conclusions about the pattern of predominant circulating strains in France, since the data reported here concern the outbreaks investigated between week 40 in 2016 and week 3 in 2017 and the gastroenteritis outbreak season is not yet over. Already, according to the phylogenetic analysis of the capsid sequences, it seems that GII.P16-GII.4 strains could be more prevalent than the GII.P4 2009-GII.4 2012 variants. Further molecular and epidemiological investigations are needed to confirm this tendency.

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Conflict of interest

None declared.

Authors' contributions

MB and LT: conducted the laboratory investigation; JK: conducted the phylogenetic analyses and revised the manuscript; ADR: revised the manuscript, KAB: compiled the data, drafted the manuscript.

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Maternal pertussis immunisation: clinical gains and epidemiological legacy

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The increase in whooping cough (pertussis) incidence in many countries with high routine vaccination coverage is alarming, with incidence in the US reaching almost 50,000 reported cases per year, reflecting incidence levels not seen since the 1950s. While the potential explanations for this resurgence remain debated, we face an urgent need to protect newborns, especially during the time window between birth and the first routine vaccination dose. Maternal immunisation has been proposed as an effective strategy for protecting neonates, who are at higher risk of severe pertussis disease and mortality. However, if maternally derived antibodies adversely affect the immunogenicity of the routine schedule, through blunting effects, we may observe a gradual degradation of herd immunity. 'Wasted' vaccines would result in an accumulation of susceptible children in the population, specifically leading to an overall increase in incidence in older age groups. In this Perspective, we discuss potential long-term epidemiological effects of maternal immunisation, as determined by possible immune interference outcomes.

Pertussis over the past 75 years

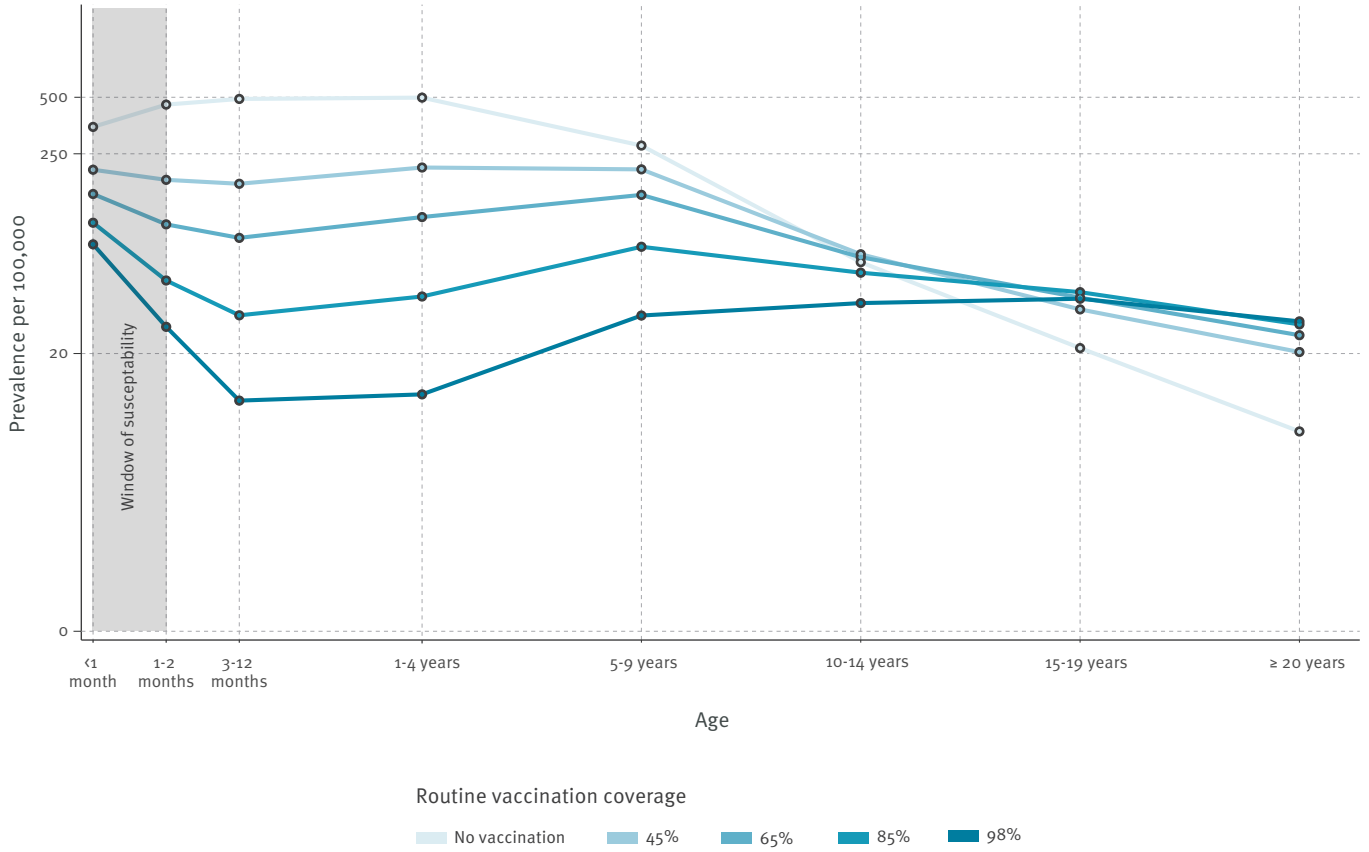
Since Jenner's time, immunisation has been a prominent instrument in the public health toolbox, especially against the microparasitic diseases of childhood. Ideally, it protects the vaccinee directly against subsequent infection or at least clinical disease [1]. Accordingly, vaccination schedules for childhood diseases have sought to reach infants as early as possible. An added bonus of transmission-blocking vaccines is the indirect protection they provide to unvaccinated individuals by reducing pathogen circulation, an effect known as herd immunity [1]. The Figure illustrates this, showing how incidence among unvaccinated infants drops as vaccine uptake increases.

Pertussis, a highly contagious childhood disease, was once considered a candidate for eradication due to the pronounced early success of immunisation in reducing morbidity and mortality in populations where high coverage was achieved [2]. In the 1940s and 1950s, a number of countries introduced routine pertussis vaccination with three doses of the whole cell vaccine (wP), delivered in infancy. The result was a marked drop in incidence and mortality including in infants too young to be immunised [2,3]. The last two decades, however, have seen pertussis incidence resurge in a number of populations where it had been under control [3]. In particular, the World Health Organization has raised concerns about the success of current vaccination strategies, following increases in pertussis incidence in some countries with long-standing high coverage, including the United States (US), the United Kingdom (UK) and Australia [2,3]. These resurgence events are characterised by increased incidence among teenagers and adults but, for the first time in decades, recent pertussis outbreaks have included infant deaths (e.g. 10 in California in 2010 and 14 in the UK in 2012) [2-4].

As yet, there is no consensus on the reasons for this resurgence. Improved diagnostics and heightened awareness appear to be partly responsible for some of the rise in incidence, but there is also clear evidence for increased bacterial circulation in these populations [3]. A variety of explanations for the latter have been proposed. These include the possibilities of (i) vaccine-driven evolution of the bacterium [5], (ii) primary vaccine failure, where some vaccinees fail to mount an immune response [6], (iii) failure of vaccines to block transmissible infection [7], (iv) increases in vaccine hesitancy [8], (v) waning of infection- and/or vaccine-induced immunity, where the loss of protection over time renders individuals susceptible [9] and (vi) gradual accumulation of susceptible individuals due to incomplete historical vaccination coverage (an 'end of honeymoon' effect) [10]. Some of these hypotheses

Figure

Illustration of how routine pertussis vaccination schedule (2, 4 and 6 months of age) affects disease prevalence by age group



Increasing vaccine coverage leads to a shift in the mean age of infection to older age groups. With an assumed basic reproduction ratio Ro of 10 and no vaccination, mean age of infection is 6 years; with vaccine coverage of 45%, 65%, 85%, and 98% the mean age of infection rises to 15, 22, 31 and 38 years, respectively. This figure was generated by numerical integration of an age-structured transmission model with age-assortative mixing [12].

link resurgence to the switch to acellular vaccines (aP) that many countries made over the past two decades in response to concerns over the reactogenicity of wP vaccines [3,11]. While the debate regarding the underlying causes of the resurgence continues, there remains an urgent need to protect newborns during the window of susceptibility, i.e. the interval between birth and the commencement of routine vaccination, which coincides with the period of maximum vulnerability to pertussis disease (Figure) [12]. During this period, immaturity of the neonate's immune system leaves the infant particularly vulnerable to complications from pertussis infection, including death [2].

Neonatal pertussis vaccination is not a viable option [2]. Because of the immaturity of the infant immune system, vaccination at too early a stage produces only a weak serological response [13]. Moreover, maternal antibodies (MatAb) can interfere with vaccination, resulting in inhibited seroconversion, a phenomenon known as 'blunting' [11]. Blunting can occur, for example, by epitope masking [14,15] and has been observed with some live vaccines (e.g. measles), where MatAb even in minute quantities can significantly inhibit seroconversion [14-16]; it is less clear whether blunting by MatAb is an actual concern in the case of pertussis. The recommended schedules for pertussis vaccination reflect these potential concerns, having been designed to prime and subsequently boost protection as the infant immune system matures and maternal antibody protection wanes [2,16].

To provide indirect protection to newborns, three main strategies have been proposed. Cocooning targets the immediate family and other likely close contacts for booster vaccination [2,4]. The second strategy aims to reduce incidence in adults and teenagers via an augmented booster schedule. The overall impact of these two strategies has been modest [2,8,14], however, leading some countries to consider a third strategy, vaccination of pregnant women, as an additional means of protecting infants [2]. The rationale is that such vaccination provides direct antenatal passive immunity via active transfer of maternal IgG, with increasing concentration of antibodies in the fetus until birth, in addition to the indirect protection as a form of cocooning [14]. Moreover, prenatal check-ups represent a convenient vehicle for such immunisations.

The case for maternal immunisation

Studies in the 1930s and 1940s established a correlation between antibody levels in mother and infant, with high titres in infants whose mothers had a history of pertussis infection or had been immunised during pregnancy [17]. Because typically fewer than 50% of pregnant women have detectable serum antibodies for pertussis [14], immunisation during pregnancy has been advocated. It is expected to result in higher neonate antibody levels, conferring clinical protection [11,14,16,17] during the window of vulnerability (Figure) [12,15]. This strategy is successfully demonstrated by maternal tetanus immunisation, which has been shown to be safe, immunogenic and protective of infants against neonatal tetanus [18].

Maternal immunisation unknowns: vaccine interference

While the motivation for maternal immunisation is clear, the need for caution in view of the potential for blunting has been noted [11,16,18-23]. To examine the risk of blunting, several studies have compared infant antibody response to the primary schedule in relation to maternal immunisation status [11,16,18-23]. In infants receiving the wP vaccine, a negative correlation was observed between MatAb titres and the immune response elicited after routine vaccination [16]. Among infants receiving the aP vaccine, however, the evidence regarding blunting effects is less clear-cut, with substantial variability between studies [11,16,20-23]. Studies of aP vaccines have variously shown reduction [20,22,23], increase [21] or no impact [16] of MatAb on the pertussis toxin-specific antibody response. The response to other antigens (filamentous haemagglutinin, fimbriae and pertactin) has been similarly inconsistent [21-23]. This discord is partly attributable to heterogeneity in study design and protocol, as well as differential vaccine histories in the included population.

Confident assessment of the epidemiological consequences of maternal immunisation is challenging both due to the aforementioned inconsistency in the findings of clinical trial studies [18] and the absence of a serological correlate for protection against pertussis. Critically, no threshold or functional relationship between antibody titres and protection is known [11,14,16,17,20-23]. Thus, the clinical or epidemiological significance of altered antibody titres remains uncertain.

A concern, therefore, is that should maternal immunisation adversely affect the strength or duration of protective vaccine-induced immunity following the primary schedule, it may ultimately give rise to higher pertussis incidence, perhaps among primary and middle school children. In a recent modelling study, we demonstrated that averting such an eventuality would require both prenatal and routine vaccination coverage to be sufficiently high [12]. Moreover, this study predicted that due to the slow rate of population turnover, such downstream increases in incidence would take decades to manifest. This phenomenon has been observed in other studies of the long-term outcomes of infection control strategies [3,10].

It is important to note that most studies of the impact of pertussis MatAb on the efficacy of the routine vaccination schedule have measured antibody responses at most one month after the administration of the third routine dose [11,16,21-23]. Studies of antibody titres after the fourth booster dose, however, found no effect of maternal immunisation history [18,20,24]. There may be two not mutually exclusive explanations for this finding: the absence of MatAb in 12–18-month-olds due to waning [12,14], and the successful boosting effect of the fourth dose, leading to antibody titres similar to control individuals.

Maternal immunisation unknowns: timing

Another aspect of maternal immunisation that warrants further research is the optimal timing of vaccination relative to pregnancy [14,19]. In newborns, MatAb levels from mothers infected or immunised before pregnancy are reduced compared with mothers immunised during pregnancy [16,17,21]. Thus, it is of practical relevance to ascertain when the most efficient transplacental transfer of antibodies occurs [14,19] as it determines the trimester during which maternal immunisation should be administered. The timing remains controversial, with newer studies proposing the second trimester of pregnancy [19], while earlier studies advised the third trimester [14].

Concluding remarks

Maternal pertussis immunisation is safe for both mother and infant [2,11] and is currently recommended in Australia, Belgium, Brazil, Portugal, the UK and the US, in response to the rise in incidence [2,18]. Its principal aim is to reduce pertussis mortality and morbidity in neonates. There is good reason to stress the direct benefits of maternal immunisation to both mother and infant. However, its potential adverse effects on routine vaccination efficacy and the subsequent long-term epidemiological legacy remain the subject of debate [11,12,16,17,20-23].

Given these unknowns, mathematical transmission models can be instrumental in predicting the magnitude and time scale of potential effects of maternal antibody interference at the population level. Our recent modelling study [12] identified a trade-off between the direct protection of infants via maternal immunisation and the reduced indirect effects of herd immunity, leading to a gradual increase in incidence among older age cohorts.

Ultimately, quantifying the efficacy and cost-effectiveness of maternal immunisation requires a two-pronged approach combining long-term clinical trials (such as the ongoing and recently finished studies in the UK, Canada and the US [18]) with epidemiological and

health economics modelling. Longitudinal clinical trials can resolve the immunological effects of MatAbs in response to routine vaccination. Furthermore, such research can shed light on the nature of any interference effect. Specifically, it is important to establish whether interference leads to an increase in vaccine failure, reduces the protective effects of the vaccine or affects the duration of protection [12,18]. By integrating information gleaned from clinical and immunological studies within epidemiological transmission models, the effectiveness of alternative strategies can be evaluated.

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Conflict of interest

None declared.

Authors' contributions

Ana I Bento wrote the first draft. Aaron King and Pejman Rohani have commented and edited the subsequent drafts. Revisions performed after editorial and reviewers' comments were made and agreed by all three authors.

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Concern regarding the alleged spread of hypervirulent lymphogranuloma venereum *Chlamydia trachomatis* strain in Europe

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To the editor: A recent surveillance and outbreak report published in *Eurosurveillance* by Petrovay et al. on the 'Emergence of the lymphogranuloma venereum L2c genovariant, Hungary, 2012 to 2016' [1] provides an observation of the first European cases of a genotype of *Chlamydia trachomatis* associated with severe haemorrhagic proctitis. The authors of this paper diagnosed the strains as lymphogranuloma venereum (LGV)-associated and performed partial sequencing of the *ompA* gene (ca 1,070 bp), which is a standard typing method for *C. trachomatis*. The *ompA* gene sequence obtained was compared with those from reference isolates, and reported to be 100% concordant with the *ompA* sequence belonging to an L2-D recombinant strain described in 2011 [2]. This strain was named 'L2c', as it was found to possess a chimeric genome, not because it has a novel *ompA*-genotype. We would like to point out that the *ompA* gene sequence of this L2-D recombinant strain, and by implication those of the Hungarian isolates, is identical to that of archetypal L2 strains, for example the reference strain L2/434 [3].

Petrovay et al. found that the *pmpH*-genotype of the Hungarian strains reflect that of an LGV strain, containing a diagnostic 36 bp deletion. Unfortunately this locus does not discriminate between L2 strains and L2-D. As the authors appear not to have checked for concordance between their strains and the L2-D recombinant strain in other genomic loci, it is not possible to determine whether the strains reflect the appearance of this L2-D recombinant, or rather a circulating L2 LGV strain. Thus, it is premature to assume that these Hungarian LGV strains reflect the presence of the 'hypervirulent' L2-D recombinant strain, despite the described clinical symptoms. We find it more likely that the authors have observed a resurgence in cases with *ompA*-genotype L2, as described last year [4].

For the *Chlamydia* community, it is important to recognise that the use of the term 'L2c genotype' in the case of the L2-D recombinant strain is a misnomer, as the *ompA*-genotype of this strain is an archetypal L2. This nomenclature was also the source of confusion in a recent paper from Slovenia describing the presence of 'L2c' [5], again with further analysis now showing that the *ompA*-genotype of this strain is also identical to L2. The distinct L2c *ompA*-genotype was described in a 2008 publication, and has 2 nucleotide differences to that of L2 [6].

Given the high level of recombination observed in *C. trachomatis* [7], typing techniques based on a few loci can never give a full indication of the underlying genomic background: only whole genome sequencing and detailed phylogenetic analysis can provide this. Therefore we would recommend that future publications are absolutely clear as to which genotyping method they have used in strain descriptions, for example a common target such as the *ompA*-genotype. Furthermore, *Chlamydia* researchers should be aware of this awkwardness of nomenclature, should thoroughly compare their *ompA* sequences against a database of known L2 *ompA*-genotypes (L2: AM884176; L2a: AB915594, L2b: AM884177; L2c: EF460796; L2d: EF460797; L2e: EF460798; L2f: EU676181; L2g: EU676180; L2bV1: JX971936; L2bV2: KU518893; L2bV3: KU518894; L2bV4: KU518892) [3,6,8-10], and report their findings more fully.

As it stands, the description of the Hungarian strains as 'L2c' is inaccurate in the sense of the *ompA*-genotype. Importantly, it is not possible to make any conclusions about the European appearance of this 'hypervirulent' L2-D recombinant strain without further sequencing of additional genomic loci, ideally whole genome sequencing, or investigations into in vitro phenotypes.

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Conflict of interest

None declared.

Authors' contributions

HSS, JCG and HdV wrote the first draft of the manuscript. DG, DAL, OP, CB, BdB, AB, IC, JK, SMB, BV, SAM, NT and AE each contributed to the draft. HdV supervised the definite version.

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Authors'reply: Concern regarding the alleged spread of hypervirulent lymphogranuloma venereum *Chlamydia trachomatis* strain in Europe

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To the editor: A recent letter by Chlamydia researchers [1] reflected on our article [2], raising a question about a potential misclassification of the published Hungarian LGV genotypes that we characterised as 'L2c'.

We agree with the authors of the letter that there is no established official nomenclature for lymphogranuloma venereum (LGV) genovariants. Moreover there are two so-called L2c variants reported in the literature, based on different typing methodologies [3,4]. The reference that we used to characterise the strains in our report was the L2c variant described by Somboonna et al. [4]. In particular, a partial sequence of the *ompA* gene of this variant was employed for typing, and, as stated in the letter, *'It is important to recognise that the use of the term 'L2c genotype' in the case of the L2-D recombinant strain is a misnomer, as the ompA-genotype of this strain is an archetypal L2'*, this sequence proved to be identical, at least at protein level, to the L2 sequence.

This fact was clarified by the LGV Genotype Dynamics Study Group, University of Basel. Due to the confusing situation of the LGV nomenclature, we have contacted this LGV research laboratory aiming to further collaborate and subtype the DNA-samples of the reported Hungarian LGV strains. We would like to point out that the Hungarian genovariants differ from the L2b variant spreading in western European countries, as was confirmed by comparison to different reference sequences of L2b sent by the LGV Genotype Dynamics Study Group, who suggested to describe the Hungarian isolates as 'L2' genovariants until further more detailed genomic analysis.

As we do not know yet whether they prove to be a new L2 type or not, we recommend to wait with the classification of these strains until we have the final typing results. We agree that only further investigation, such as whole genomic sequencing and phylogenetic analysis can confirm the genomic background and these techniques may reveal some misnomers of LGV genotypes reported previously in other publications.

Conflict of interest

None declared.

Authors' contributions

FP, EB and TE prepared the letter collectively.

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