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Rapid communications

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 [3]. 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
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, ceftriaxone, 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.
K. pneumoniae bapenemases in Ireland were identified in 2016. In 2016, the three most commonly reported carbapenemases in Enterobacteriaceae include KPC (carbapenem-hydrolysing oxacillinase-48), OXA-48, and NDM. 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...

**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 XbaI. Isolates are between 83% and 97% similar by PFGE.
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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