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High prevalence of *Legionella* in non-passenger merchant vessels

S. L. COLLINS[1]*, D. STEVENSON[1], M. MENTASTI[2], A. SHAW[3], A. JOHNSON[4], L. CROSSLEY[5] AND C. WILLIS[6]

[1] *Biosafety, Air and Water Microbiology Group, National Infection Service, Public Health England, Porton, UK*
[2] *Respiratory and Vaccine Preventable Bacteria Reference Unit, Public Health England, Colindale, London, UK*
[3] *Northern Ireland Public Health Laboratory, Belfast Health and Social Care Trust, Belfast City Hospital, Belfast, UK*
[4] *Food, Water and Environmental Microbiology Laboratory, National Infection Service, Public Health England, Colindale, London, UK*
[5] *Manchester Port Health Authority, Runcorn, Cheshire, UK*
[6] *Food, Water and Environmental Microbiology Laboratory, National Infection Service, Public Health England, Porton, UK*

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SUMMARY

There is a paucity of information on the risk from potable water in non-passenger merchant vessels (NPMVs) particularly with regard to *Legionella* and other bacteria. This retrospective study examined water samples from 550 NPMVs docked in eight UK ports. A total of 1027 samples from 412 NPMVs were examined for total aerobic colony counts (ACC), coliforms, *Escherichia coli* and enterococci; 41% of samples yielded ACC above the action level ($>1 \times 10^3$ c.f.u./ml) and 4·5% contained actionable levels (>1 c.f.u./100 ml) of faecal indicator bacteria. Eight hundred and three samples from 360 NPMVs were cultured specifically for *Legionella* and 58% of vessels proved positive for these organisms with 27% of samples showing levels greater than the UK upper action limit of 1×10^3 c.f.u./l. Cabin showers (49%) and hospital shower (45%) were frequently positive. A subset of 106 samples was analysed by quantitative polymerase chain reaction for *Legionella* and identified a further 11 *Legionella*-positive NPMVs, returning a negative predictive value of 100%. There was no correlation between NPMV age or size and any microbial parameters ($P > 0{\cdot}05$). *Legionella pneumophila* serogroup 1 was isolated from 46% of NPMVs and sequence-based typing of 17 isolates revealed four sequence types (STs) previously associated with human disease. These data raise significant concerns regarding the management of microbial and *Legionella* risks on board NPMVs and suggest that better guidance and compliance are required to improve control.

Key words: *Legionella*, water-borne infections, water (safe), water (quality).

INTRODUCTION

Since the middle ages ships have played a major role in the global transmission of disease including plague and cholera [1]. Today, the international merchant shipping industry comprises more than 100 000 sea-going vessels and about 55 000 of

* Author for correspondence: Dr S. L. Collins, Biosafety, Air and Water Microbiology Group, National Infection Service, Public Health England, Porton, SP4 0JG, UK.
(Email: Samuel.collins@phe.gov.uk)

these are non-passenger merchant vessels (NPMVs) [2].

The provision of safe, potable water on board ships is critical for the health of passengers and crew alike. This can be challenging as vessel water systems may be complex and contamination can occur at multiple points during supply, bunkering, storage and use [1]. Between 1970 and 2000 the World Health Organization (WHO) attributed one fifth of ship-associated outbreaks to contaminated water [3] and a number are well documented [4]. The revised edition of the WHO guidelines [1] recognizes that the risk of waterborne outbreaks on board ships can be mitigated through the appropriate handling of potable water and the maintenance of water distribution systems. Water safety plans, encompassing the principles of Hazard Analysis and Critical Control Points are the recommended means of achieving this. These are based on a three-stage model; risk assessment, control measures and management procedures [5].

Legionellae are ubiquitous in freshwater and thrive in man-made water systems operating between 20 °C and 45 °C. They are the causative agents of two clinical syndromes, Legionnaires' disease (LD), a severe pneumonia, and Pontiac fever, a self-limiting 'flu-like' illness [6]. Infection typically follows the inhalation of contaminated aerosols from sources including cooling towers, spa pools and hot- and cold-water systems [7]. Worldwide, the incidence of LD is increasing with the highest number of reported cases ever in Europe in 2014 [8]. The WHO provides guidance on managing the risks associated with *Legionella* on board merchant vessels [1, 9]. Despite this and other country-specific guidance [10, 11] studies have shown a high prevalence of *Legionella* contamination in passenger vessels [12, 13]. Between 1977 and 2012 there were ~200 cases and >10 fatalities due to LD associated with ships [3, 14, 15]. The majority of cases occurred in cruise ships and included outbreaks on a Rhine cruise in 1997 [14], a Nordic cruise in 2003 [16] and a UK cruise ship in 2007 [17]. Thirteen cases were associated with NPMVs including an outbreak linked to a cargo vessel undergoing repair [18] and more recently, a recurrent *Legionella* outbreak has been reported in a Norwegian NPMV [19]. In 1993, a survey of yachts showed that ~40% had culturable *Legionella* from water systems at concentrations of 10^2–10^3 c.f.u./l [20] and a study of Norwegian naval vessels detected *Legionella* by quantitative polymerase chain reaction (qPCR) and culture in 20/41 vessels and showed that contamination most likely originated from the port bunkering station [21].

A study by Grenfell *et al*. in 2005 [22] examined the microbiological quality of potable water (but not *Legionella*) on cargo vessels docking at UK ports and found that 8·6% of samples contained coliforms, *Escherichia coli* or enterococci and 20% had aerobic colony counts (ACCs) above actionable levels. However, to our knowledge, there are no published studies on the prevalence of *Legionella* in NPMVs. This is an important data gap as such vessels constitute ~50% of all seafaring traffic and are manned by in excess of 1·2 million crew [2].

The UK Merchant Shipping (Provisions and Water) Regulations 1989 [23] state that it is the duty of employers and masters of ships to supply water free from organisms likely to cause harm. Merchant vessels docking at UK ports are subject to inspection by the Port Health Authority (PHA) under the European Union directive on Port State Control [24]. Water samples may be taken to ensure compliance with UK and international standards and as a means of validating risk management.

The control of *Legionella* in the UK is supported by the Health and Safety Executive (HSE) Approved Code of Practice (ACoP) L8 and Health and Safety Guidance HSG 274 part 2 [11] which provides action and alert levels used by Port Health Officers to interpret results of *Legionella* testing and to recommend remedial actions. The lower and upper action limits dictated by this guidance are 1×10^2 and 1×10^3 c.f.u./l, respectively. Results greater than the upper action limit necessitate an immediate review of the risk assessment and control measures to identify remedial action including possible disinfection. Re-testing should occur at regular intervals thereafter until satisfactory control (results below the lower action limit) has been achieved.

This retrospective study analysed microbiological test results of potable water samples collected from NPMVs docked at UK ports between 2013 and 2016. To our knowledge this is the first report on the prevalence of *Legionella* in NPMVs.

MATERIALS AND METHODS

Sample collection

Samples were collected from 550 NPMVs between April 2013 and February 2016 during routine inspections by PHAs. Vessels were docked in the

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ports of Belfast, Bristol, Falmouth, Fowey, Manchester, Plymouth, Southampton and Weymouth. For *Legionella*, 803 1-litre water samples were taken from 360 NPMVs. Samples were taken without flushing and in accordance with BS7592:2008 [25] using sterile plastic bottles. Showers were chosen as representative points due to ease of access, risk presented to crew/passengers and as they are usually a mixture of both hot and cold water. Samples from showers were taken using a sterile bag wrapped around the shower head to funnel water into the sample bottle while minimizing aerosol generation.

Samples for ACCs, total coliforms and faecal indicators (*E. coli* and enterococci) ($n = 1027$) were obtained from 412 NPMVs using 500 ml bottles in accordance with Public Health England (PHE, formerly Health Protection Agency) guidelines [26]. Galley and bridge taps were frequently sampled as these are regularly used by the crew, easily accessible and the bridge tap usually represents a distal point of the water distribution system. All samples were tested for ACCs and 985 were tested for coliforms and faecal indicators at the request of the submitting PHA. All sample bottles contained 20 mg/l sodium thiosulphate to neutralize oxidizing biocides. They were transported to the laboratory under controlled conditions and examined within 24 h for ACCs, coliforms and faecal indicators and within 48 h for *Legionella*.

Sample analysis by culture

Samples were analysed at two PHE Food, Water and Environmental laboratories and the Northern Ireland Public Health Laboratory, Belfast. *Legionella* samples were processed according to ISO 11 731:1998 [27]. Briefly, 1 litre of water was concentrated by membrane filtration and bacteria were re-suspended in 10 ml 1/40 Ringers solution. Sample concentrates (500 µl) were cultured on glycine-vancomycin-polymyxin-cycloheximide (GVPC) agar (Oxoid, UK) either directly (untreated), or after additional heat (30 min at 50 °C) or acid (0·2 mol/l HCl-KCl, pH 2·2 for 5 min) treatments. Cultures were incubated at 36 ± 1 °C for 10 days. *Legionella*-like colonies, exhibiting cysteine auxotrophy, were further identified to *Legionella pneumophila* serogroup 1 (sg1), *L. pneumophila* sg2–14 or *Legionella* spp. by latex agglutination (ThermoFisher Scientific, UK). The limit of detection differed between testing laboratories but was either 20 or 100 c.f.u./l. Results were interpreted according to HSE guidelines [11]. ACC analysis was conducted by the pour plate method as described previously [28] and duplicate plates were incubated at 22° C for 68 ± 4 h and 37 °C for 44 ± 4 h. Total coliforms and *E. coli* were determined by a most probable number (MPN) method using Quanti-Tray™ (IDEXX, UK) according to the manufacturer's instructions. Enterococci were examined by membrane filtration according to a standard method [29] and cultures were incubated at 30 °C for 4 ± 1 h, followed by 37 °C for 40 ± 4 h; presumptive enterococci were confirmed by the aesculin hydrolysis test. ACCs, coliforms, *E. coli* and enterococci results were interpreted with reference to PHE guidelines [26]. Results were deemed unsatisfactory if counts exceeded the action limits of 1×10^3 c.f.u./ml for ACCs and 1 c.f.u./100 ml for coliforms, *E. coli* and enterococci.

Sample analysis by qPCR

Between June 2014 and March 2015 *Legionella* samples were also examined by qPCR as part of an evaluation of qPCR for all water samples submitted to one of the laboratories. DNA was extracted from sample concentrates and a duplex qPCR for *Legionella* spp. and *L. pneumophila* performed as described previously [30] with the following modification: TaqMan® Fast Environmental Master Mix Beads (ThermoFisher) incorporating a VIC 3'-labelled internal positive control were used for all qPCR reactions. The limits of detection (LOD) and quantification were 83 genome units (GU)/l and 166 GU/l, respectively. Data were analysed using ABI 7500 v. 2.3 Software (ThermoFisher).

Sequence-based typing (SBT)

SBT of 17 *L. pneumophila* sg1 isolates, obtained from 17 individual NPMVs between 2013 and 2016 was conducted according to an internationally validated method [31, 32]. Results were analysed using the online Sequence Quality Tool [33] to obtain allelic profiles and sequence types (STs). Alleles that could not be assessed by SBT were marked as '0'.

Statistical analysis

Data were analysed using Microsoft Excel 2010 (Microsoft Corp., USA) and SigmaStat v. 4.0

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(Systat Software Inc., USA). The Mann–Whitney *U* test was used to compare medians. Linear regression analysis was used to compare ship's age and *Legionella* culture results. Differences were considered statistically significant when $P < 0{\cdot}05$.

RESULTS

NPMVs

The majority of NPMVs were cargo vessels, including bulk and container carriers (48·6%, $n = 268$), chemical/oil product tankers (23·1%, $n = 129$) and a small number of dual cargo/passenger vessels (7·3%, $n = 40$). Other vessels included dredgers, cable layers, tugs, research ships and pleasure craft. Ships were aged between 1 and 79 years and sized between 40 and 163 882 gross tonnage (GT) (Table 1). The majority of vessels (77·5%, $n = 427$) were registered in countries other than the UK and 87% ($n = 481$) undertook international routes.

Microbiological quality of potable water

On average, 2·5 samples were obtained from 412 vessels. Samples were taken from galley taps ($n = 511$), other potable water sources including the bridge and cabins ($n = 412$), drinking water dispensers ($n = 44$), showers ($n = 41$), water storage tanks ($n = 12$), ice machines and evaporators ($n = 7$).

For ACCs, results ranged from 0 to $2{\cdot}08 \times 10^5$ c.f.u./ml (median 37) and 0 to $9{\cdot}6 \times 10^5$ c.f.u./ml (median 84) for 37 °C and 22 °C, respectively; 41% (422/1027) of samples yielded $>1 \times 10^3$ c.f.u./ml. The proportion of unsatisfactory samples ($>1 \times 10^3$ c.f.u./ml) was slightly, but not significantly, higher at 37 °C than at 22 °C (Table 2). Actionable levels at 37 °C (indicating a possible deterioration in potable water quality) [34] were most frequent in drinking water dispensers (31·8%, $n = 14$), other potable water taps (21·1%, $n = 87$) and galley taps (19·2%, $n = 98$); 39% ($n = 160$) of vessels had one or more unsatisfactory samples. The median age of these vessels was not significant compared to all vessels sampled ($P = 0{\cdot}42$) (Table 1). Cargo vessels were more frequently associated with actionable samples (44·3%, $n = 71$), followed by chemical/oil product tankers (19·3%, $n = 31$).

For coliforms, 4% ($n = 39$) of samples were unsatisfactory (>1 c.f.u./100 ml), representing 34 individual vessels (Table 2) and counts ranged from 1 to >201 MPN/100 ml. The majority of positive samples were obtained from galley taps (78%, $n = 32$) followed by other potable water taps (17·1%, $n = 7$) and one drinking water dispenser (2·4%). Cargo ships had the highest rate of failure (50%, $n = 20$) followed by chemical/oil product tankers (20%, $n = 8$). Neither the median age nor size of coliform-positive vessels were significant ($P = 0{\cdot}16$ and $P = 0{\cdot}31$, respectively) compared to all the vessels sampled; 74% were registered in countries other than the UK and all undertook international routes (Table 1). One sample (0·1%) from a galley tap was positive for *E. coli*. Four vessels (0·97%) had unsatisfactory levels of enterococci (>1 c.f.u./100 ml) recovered from three galley taps and one other potable water tap with counts up to 7 c.f.u./100 ml (Table 2). Eleven (27·5%) samples positive for coliforms, *E. coli* or enterococci also gave unsatisfactory ACC results.

Legionella

Eight hundred and three samples were analysed for *Legionella* from 360 vessels. Some ($n = 198$) were sampled at least twice either on the same day or on separate dockings. The majority of samples (73·3%, $n = 589$) were obtained from crew and cabin showers, 13·6% ($n = 109$) from other potable hot-water taps, 9·1% ($n = 73$) from hospital showers, and 4% ($n = 32$) from other sources including hydrophores and air-conditioning units. In total 48·6% ($n = 391$) were positive for *Legionella* by culture (Table 3) at concentrations from 20 to $1{\cdot}8 \times 10^6$ c.f.u./l (median $1{\cdot}76 \times 10^3$ c.f.u./l), representing 58·3% ($n = 210$) vessels. Forty-seven per cent of samples contained *L. pneumophila* sg2–14 and 36% *L. pneumophila* sg1, representing 53·8% and 46·7% of positive vessels, respectively. *Legionella* species other than *L. pneumophila* were also isolated and 11% of samples had mixed *Legionella* populations. Sixty per cent of vessels ($n = 107$) were positive from at least two different outlets. Neither the median age nor median size of *Legionella*-positive vessels was significant compared to all the vessels sampled ($P = 0{\cdot}48$ and $P = 0{\cdot}32$, respectively) (Table 1). There was no relationship between ship's age and *Legionella* culture result (c.f.u./l) ($R^2 = 0{\cdot}0008$, $P = 0{\cdot}57$).

According to UK guidance, 44·4% ($n = 357$) of samples would have required action by the vessel operator; 28% ($n = 224$) yielded counts greater than the upper action limit of 1×10^3 c.f.u./l and 8·2% ($n = 66$) were $>1 \times 10^4$ c.f.u./l. The majority of these samples were obtained from cabin and hospital

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Table 1. *Characteristics of NPMVs sampled for ACC, coliforms, faecal indicators or* Legionella

	NPMVs				
	Sampled for ACC, coliforms and faecal indicators	>action level for ACC	>action level for coliforms or faecal indicators	Sampled for *Legionella*	Positive for *Legionella*
Median age (years) (95% CI)	13 (12–13)	13·5 (13–14)	16·5 (14–22)	13 (12–14)	13 (13–14)
Median size (GT) (95% CI)	4473 (2999–9980)	3440 (267– 43 717)	3978 (2829–9981)	4987 (4151–6118)	5006 (4235–7087)
UK registration (%)	22·5	32·5	26	20·5	22·4
International routes (%)	87	81	100	93	94

NPMVs, Non-passenger merchant vessels; ACC, aerobic colony count; CI, confidence interval; GT, gross tonnage.

Table 2. *Samples and NPMVs positive for ACC, coliforms,* E. coli *and enterococci with respect to published guidelines*

	ACC 22 °C	ACC 37 °C	Coliforms	*E. coli*	Enterococci
No. samples >action level* (%)	204/1027 (19·9)	218/1027 (21·2)	39/985 (4·0)	1/985 (0·1)	4/985 (0·4)
No. vessels >action level (%)	160/412 (38·8)	160/412 (38·8)	34/412 (8·3)	1/412 (0·24)	4/412 (0·97)

NPMVs, Non-passenger merchant vessels; ACC, aerobic colony count.
* Action levels are $>1 \times 10^3$ c.f.u./ml for ACC and >1 c.f.u./100 ml coliforms, *E. coli* and enterococci.

Table 3. Legionella *culture results of water samples and percentage of NPMVs positive for* Legionella *above action limits*

Sampling point	No. samples	Positive *n* (%)	>lower limit* *n* (%)	>upper limit* *n* (%)
Cabin shower	589	290 (49·2)	103 (17·5)	161 (27·3)
Tap	109	62 (56·8)	19 (17·4)	37 (33·9)
Hospital shower	73	33 (45·2)	11 (15·1)	22 (30·1)
Other	32	6 (18·8)	0 (0·0)	4 (13·3)
Total	803	391 (48·6)	133 (16·5)	224 (27·9)
% vessels		58·3	50·2	43·3

NPMVs, Non-passenger merchant vessels.
* UK lower (1×10^2 c.f.u./l) and upper (1×10^3 c.f.u./l) action levels [11].

showers (Table 3). Other positive sites ($>1 \times 10^3$ c.f.u./l) included a hydrophore and three air-conditioning units on four separate vessels. Cargo vessels were most frequently positive (54·2%, $n = 114$) followed by chemical/oil product tankers (26·6%, $n = 56$). The majority of *Legionella*-positive vessels were registered in countries other than the UK (Table 1). Of the *Legionella*-positive vessels that were also sampled for ACCs, 8% (13/162) were associated with results $>1 \times 10^3$ c.f.u./ml. Most (94%, $n = 197$) vessels undertook international routes.

One hundred and six samples (15·9%) were also tested by *Legionella* qPCR. *Legionella* was detected (GU/l >LOD) in 75·5% of samples ($n = 80$) at concentrations between $2{\cdot}16 \times 10^2$ and $5{\cdot}09 \times 10^6$ GU/l (median $2{\cdot}05 \times 10^4$ GU/l) and $1{\cdot}8 \times 10^2$ and $2{\cdot}23 \times 10^5$ GU/l (median $8{\cdot}3 \times 10^3$ GU/l) for *Legionella* spp. and *L. pneumophila*, respectively. Thirty-eight per cent ($n = 41$) of samples, and 11 vessels, respectively, which were negative by culture were positive by qPCR. There were no instances of culture-positive–qPCR-negative results, indicating a qPCR negative predictive value of 100% (95% confidence interval 96·6–100).

SBT

Complete profiles and corresponding STs were obtained for 12/17 isolates. Partial profiles were obtained for the remaining five (Table 4).

DISCUSSION

In the UK, PHAs are responsible for inspecting and sampling water systems on board NPMVs. Between 2013 and 2016, samples from NPMVs docked in

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Table 4. *SBT results for 17* L. pneumophila *sg1 isolates obtained from NPMVs (11 cargo, 4 research, 2 naval, 1 oil tanker)*

SBT allele profile	Sequence type	No. of NPMVs	Clinical isolates in SBT database	Countries of isolation‡
0,14,16,16,15,13,2	0*	5	16†	CAN, CHN, FRA, GBR, ITA, JPN, KWT NLD, NOR, USA
1,4,3,1,1,1,1	1	9	757	Worldwide
1,4,3,1,1,1,3	152	1	2	DEU, FRA, GBR, ZAF
3,10,1,28,14,9,3	187	1	13	CAN, CHE, DEU, GBR, ISR, MAL, NLD, SIN, SWE, USA
3,13,1,3,14,9,11	579	1	3	BEL, CHN, DEU, FRA, ITA, LVA, NOR

SBT, Sequence-based typing; NPMVs, non-passenger merchant vessels.
* Partial allelic profile only, consistent with ST154.
† Isolates of ST154.
‡ Three-letter country codes according to ISO 3166.

eight UK ports were analysed for microbiological parameters including *Legionella*. The low prevalence of coliforms or faecal indicators represents an improvement compared to a previous study [22]. Although there were a limited number of positive NPMVs there was no association between NPMV age and the risk of potable water containing coliforms, *E. coli* or enterococci. Forty per cent of NPMVs had one or more ACC result above the action limit indicating a potential deterioration in water quality. According to PHE, guidelines [26] $>1 \times 10^3$ c.f.u./ml ACC in the absence of other indicators would necessitate a hyper-chlorination and refilling of the potable water system at considerable cost to the vessel. ACCs can provide actionable results to PHAs while a vessel is docked; however, interpretation is difficult particularly when considering an individual result in the absence of ongoing trend data. Bacterial numbers will gradually increase in stored water, and high ACCs do not necessarily equate to a public health risk [35]. In this study actionable ACCs did not correlate with coliforms, *E. coli*, enterococci or *Legionella* failures. These data are in agreement with Grenfell *et al.* [22] and suggest the value of one-off ACC sampling should be reconsidered and public health resources focused on monitoring faecal indicators and *Legionella*. ACCs will remain a useful measure of disinfection efficacy but should only be used to routinely monitor potable water when trend data is available.

High prevalence of *Legionella* in passenger vessels has previously been described [12, 13]. This study is the first to report on the prevalence of *Legionella* in NPMVs. Over half of NPMVs were positive for *Legionella*. There was no association between *Legionella* prevalence and the age or size of the NPMV. The finding that 28% of shower samples were greater than the upper action limit of 1×10^3 c.f.u./l is concerning given the aerosol potential of showers and their association with LD [36–38]. Of particular concern is the high prevalence of *Legionella* in ships' hospital showers with approximately half testing positive by either culture or qPCR, and 30% of culture positives being above the upper action limit. Hospital showers by their very nature may be used by individuals who are more at risk from exposure to *Legionella*. Simple procedures such as regular cleaning and descaling of heads and hoses as recommended by UK and WHO guidance [1, 11] can help mitigate the risk of *Legionella* from shower systems or alternatively, heads and hoses can be removed, disinfected, dried and stored. Low-use outlets and showers (particularly hospital showers) should be identified in the risk assessment and regularly flushed to avoid water stagnation.

L. pneumophila is responsible for ~90% of LD and serogroup 1 is the most prevalent disease causing serogroup [39]. Ship-borne outbreaks of *L. pneumophila* other than serogroup 1 have been reported [14, 17] therefore detection of non-serogroup 1 strains is important and should be used as an indicator that serogroup 1 could be present. Forty-six per cent of *Legionella*-positive NPMVs were positive for *L. pneumophila* sg1. To gain an insight into the distribution of *L. pneumophila* sg1 STs, available isolates from 17 NPMVs were analysed according to the international SBT scheme. Although four complete ST profiles were found, for five isolates, a complete ST profile could

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not be obtained due to failure to amplify the *flaA* gene. However, the partial profile was consistent with ST 154 (11,14,16,16,15,3,2) and previous whole genome sequencing of similar isolates has shown them to carry *flaA* 11 (PHE, unpublished data). Each of the STs identified are geographically dispersed across the world (Table 4). ST1 for example, accounted for 9/17 (53%) isolates and historically represents a significant burden (~10%) of culture-proven LD in England and France [40]; there are 757 clinical ST1 isolates out of a total of 7198 listed in the international SBT database [33]. Due to the retrospective nature of this study, relatively few *L. pneumophila* sg1 isolates were available for SBT but nonetheless this is an interesting finding and suggests that a proportion of NPMVs are colonized with STs previously associated with human disease. To further investigate ST distribution, it would be advantageous to sequence more isolates including multiple representatives from the same vessel and to correlate them with other factors such as where the vessel last bunkered water, travel routes, *Legionella* control and water-treatment methods.

A recent study by David *et al.* [41] demonstrated that the five major disease-causing clones of *L. pneumophila*, including ST1 have recently evolved and spread rapidly across the globe by an as yet unknown mechanism. The majority of NPMVs positive for *Legionella* in this study undertook international routes and thus such vessels could facilitate the spread of *Legionella* isolates around the world, a hypothesis that warrants further research.

qPCR has previously been used as a screening tool for environmental samples including ship waters [21, 30]. In this study qPCR was more sensitive compared to culture, detecting *Legionella* in an additional 41 samples and 11 NPMVs that were negative by culture. The qPCR positivity rate for ship waters was higher than for any other water system (cooling towers, hot and cold, spa pools) examined in the same period (PHE, unpublished data). Reasons for the discrepancy between culture and qPCR are well documented [30] and include inhibition of *Legionella* by other microorganisms as well as the detection of dead or viable but non-culturable cells. Despite the potential to detect dead cells, the high GU/l values for some NPMVs suggest active contamination that warrants further investigation and the 100% negative predictive value of the assay could allow specific and rapid screening of samples. Likewise, the detection of high concentrations of *Legionella* DNA could be used as a means by which PHAs can recommend remedial action in a timely manner which is not possible by culture alone.

Forty-four per cent of *Legionella* samples required action to be taken based on UK guidance [11]. As a minimum this should include flushing and resampling of the implicated outlet as well as a review of the risk assessment and control measures. However, it can be difficult for PHAs to enforce this as they have limited powers on board vessels. Furthermore, the average turnaround time of a vessel in port is 17·4 h [42] meaning the vessel is likely to have left before test results are received. This highlights the importance of port-to-port communication and International Health Requirements for informing next ports of call for the remedial actions that need to be taken.

The majority of UK seafarers are male and 52% are aged >40 years [43]. A study of French seafarers showed that 44% were smokers and 11% drank alcohol every day [44]. Male gender, age, smoking and alcohol consumption are recognized risk factors for LD [6]. In 1996, Temeshnikova *et al.* reported that 29% of cargo vessel crews had serum antibody titres >1:128 to *L. pneumophila* sg1 [45] compared to 1–20% of the general population. Together with the results in this study these data suggest that merchant seafarers may be more frequently exposed to *Legionella*. Nonetheless, UK surveillance data have not recorded any cases of legionellosis in UK merchant seafarers since 1987 and to our knowledge there is only one report of legionellosis associated with a NPMV since 2001 [19]. It is possible that cases are under-reported. Vessels stay at sea for long periods and dock in multiple countries. Seafarers with, for example, Pontiac fever may not present to medical authorities and those that do may be taken ashore and repatriated. If, for example, this falls outside the geographical area of the European surveillance system ELDSnet [46] cases may not be reported. There is therefore an opportunity to improve surveillance of infections in NPMVs and any future guidance should take this into account.

This study has some limitations. A small number of samples were taken from each NPMV and thus it is not possible to conclude with accuracy the extent of *Legionella* contamination in each NPMV (e.g. local or systemic). However, of the positive vessels, 60% were positive from at least two different water outlets indicating likely systemic colonization. The retrospective nature of this study meant that it was not possible to capture detailed information about the water systems and control measures on board each

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NPMV. However, such data would help to interpret *Legionella* results and reveal factors that might promote or protect against colonization with these organisms. This would provide evidence for future guidance and may assist PHAs in devising a risk assessment-based inspection protocol to help prioritize limited public health resources.

Coliforms and faecal indicator results showed the general quality of potable water on board NPMVs had improved in recent years. However, it is clear that further actions are required by operators of NPMVs to control the risk from *Legionella*. Water safety plans have been demonstrated to be a promising tool to prevent waterborne illness on vessels [47] and such plans must contain provisions for the control of *Legionella.* There are several guidance documents available [1, 5, 10, 11] which contain information pertinent to water quality on NPMVs but based on our findings we recommend that current guidelines are strengthened with regard to the control of *Legionella* in these vessels. Such guidelines should also incorporate a recommendation to cease one-off ACC sampling and focus resources on more useful indicator organisms and *Legionella*.

In conclusion, this study is the first to describe the prevalence of *Legionella* in NPMVs. Vessels were frequently positive and a large proportion of results were greater than the UK upper action limit. SBT indicated that some NPMVs were contaminated with *L. pneumophila* sg1 STs previously associated with human disease. This presents a risk of infection to merchant seafarers and raises significant concerns about the management of *Legionella* on board NPMVs. This study provides data that could be used to support an industry-wide evaluation of *Legionella* contamination and control on NPMVs with a view to informing future guidance and increasing awareness and compliance in the international merchant shipping industry.

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DECLARATION OF INTEREST

None.

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