**Aetiology, source and prevention of waterborne healthcare-associated infections: a review**

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The purpose of this review is to discuss the scientific literature on waterborne healthcare-associated infections (HCAIs) published from 1990 to 2012. The review focuses on aquatic bacteria and describes both outbreaks and single cases in relation to patient characteristics, the settings and contaminated sources. An overview of diagnostic methods and environmental investigations is summarized in order to provide guidance for future case investigations. Lastly, on the basis of the prevention and control measures adopted, information and recommendations are given. A total of 125 reports were included, 41 describing hospitalized children. All cases were sustained by opportunistic pathogens, mainly *Legionellaceae*, *Pseudomonadaceae* and *Burkholderiaceae*. Hot-water distribution systems were the primary source of legionnaires’ disease, bottled water was mainly colonized by *Pseudomonaceae*, and *Burkholderiaceae* were the leading cause of distilled and sterile water contamination. The intensive care unit was the most frequently involved setting, but patient characteristics were the main risk factor, independent of the ward. As it is difficult to avoid water contamination by microbes and disinfection treatments may be insufficient to control the risk of infection, a proactive preventive plan should be put in place. Nursing staff should pay special attention to children and immunosuppressed patients in terms of tap-water exposure and also their personal hygiene, and should regularly use sterile water for rinsing/cleaning devices.

**Introduction**

Healthcare-associated infections (HCAIs) are defined as infections occurring during or after the process of care that are not present or incubating at the time of the patient’s admission to a hospital or other healthcare facility. By reviewing scientific literature from 1995 to 2010, the World Health Organization estimated the overall frequency of HCAIs in developed countries as between 5.1 and 11.6% (WHO, 2011). The impact of HCAIs involves prolonged hospital stay, long-term disability, increased resistance of micro-organisms to antimicrobials, additional financial burden to health systems, high costs for patients and their family, and unnecessary deaths.

Among the many sources of infection, water should be considered of particular relevance due to the numerous occasions of exposure (Anaissie et al., 2002). In addition to drinking, potable water serves many functions in the healthcare environment such as sanitation, ventilation and air conditioning, ice production, patient bathing, physiotherapy and water birth pools, cleaning and reprocessing of medical devices (Williams et al., 2013). Various micro-organisms are natural inhabitants of potable water, favoured by biofilm formation, stagnation, corrosion and aged distribution systems (Borella et al., 2005; Williams et al., 2013). Bacteria associated with waterborne nosocomial infections mainly include aerobic Gram-negative bacilli and non-tuberculous mycobacteria; in some reports, fungal and viral pathogens are also implicated (Anaissie et al., 2002; Merlani & Francioli, 2003; Exner et al., 2005; Decker & Palmore, 2013). The majority of bacteria are opportunistic and thus immunocompromised patients, such as people of advanced age or with cancer, leukaemia, human immunodeficiency virus infection, diabetes and transplantation, are the most susceptible to contracting a severe infection after water contact (Anaissie et al., 2002). For these patients, neither implementation of guidelines nor application of water treatments appears sufficient to eliminate the risk of water HCAI (Sheffer et al., 2005).

The aim of the present study was to review the characteristics of water-associated HCAIs described in the scientific literature from 1990 to 2012. The review focuses on the aquatic bacteria responsible for HCAIs and describes both outbreaks and single cases in relation to patient characteristics, settings and contaminated sources. All types of water used in healthcare structures, such as drinking, distilled, sterile and bottled water, were considered. An
overview of diagnostic methods and environmental investigations is summarized in order to provide a helpful contribution to case management. Lastly, information about prevention and control measures adopted are given, together with recommendations addressed to healthcare personnel involved in HCAI risk assessment and management.

Methods
We reviewed the literature in PubMed by combining the bacterial name plus ‘waterborne’ and one or more of the following terms: ‘outbreak’, ‘case report’, ‘infection’, ‘nosocomial’ and ‘hospital-acquired’. We also associated the pathogen name with the setting of appearance, such as ‘intensive care’, ‘dialysis’, ‘onco-haematological’, ‘burn unit’ and ‘paediatric unit’. Other articles were searched for on the Outbreak Database (http://www.outbreak-database.com). References cited in the selected articles were used to identify additional reports. Only articles on bacterial infections written in English from 1990 to 2012 were considered. Pseudo-infections and pseudo-outbreaks, as defined by the authors themselves, and articles not specifying the number of patients were excluded. A checklist was produced to draw from each article the following information: reference, year and country, number of cases, type of micro-organism, Legionella species and serogroups, type of patient and setting, source of infection, risk factors, diagnostic and environmental investigations, and preventive measures. Bacterial species have been gathered in genera to simplify the tables. This classification was done by consulting the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/taxonomy) and the relevant scientific literature.

Results
Aetiology of waterborne nosocomial infections
Table 1 lists 125 articles describing waterborne HCAIs that occurred from 1990 to 2012 in chronological order and subdivided according to the bacterial family. All the pathogens were opportunistic environmental bacteria, mainly Legionellaceae (38.4 %) responsible for pneumonia, Pseudomonadaceae (19.2 %) generally related to bloodstream and pneumonia/respiratory tract infections, and Burkholderiaceae (12.8 %) mostly associated with bloodstream infections. Among the Legionellaceae, Legionella pneumophila serogroup 1 was responsible for cases in 32 out of 48 articles (66.7 %) followed by serogroups 6 (14.6 %), 3 (10.4 %) and 5 (6.2 %). The number of articles involving Legionella spp. was substantially stable over the years (26 in 1990–2000 and 22 in 2001–2012), but the articles describing two or more cases in the same structure were halved (21 vs 10) and the number of patients decreased (187 vs 66). Both the number of articles and cases due to Pseudomonadaceae, Burkholderiaceae, Mycobacteriaceae and Enterobacteriaceae were instead higher between 2001 and 2012 (38 articles and 1359 cases) than between 1990 and 2000 (18 articles and 181 cases).

European and American countries accounted for 52.8 and 28.8 % of articles, respectively, with rare episodes described in other continents. The largest number were from the USA (28 articles), France (14 articles) and Germany (11 articles).

Diagnostic investigation
In water HCAIs not involving Legionellaceae (77 articles), the diagnostic investigation to isolate the strain responsible was carried out by culture from blood, respiratory specimens, urine and stool. In the case of legionnaires’ disease, culture, which is necessary for strain isolation, is often combined with other laboratory diagnostic methods such as urine antigen detection, serological tests, PCR and direct fluorescence (Table 2). Culture combined with both Legionella urinary antigen and serology or with urine antigen alone, is the most frequently used approach to diagnosis. Direct fluorescence was mainly used before 2000, whilst the PCR-based methods were introduced after 2006. The use of serology and urine antigen detection remained constant over the years.

Of the three articles that did not perform culture analysis, two reported the unavailability of samples for culture (Franzin et al., 2001; Haupt et al., 2012), whereas Benz-Lemoine et al. (1991) performed only direct immunofluorescence and serology. In six articles, the diagnostic tests used were not reported (Levin et al., 1991; Mastro et al., 1991; Struelens et al., 1992; Graman et al., 1997; Borau et al., 2000; TercéI-Zorman et al., 2004).

The number and percentage of positive results for each diagnostic test were similar for the cultural method (79.3 %, 96 positive results out of 121 cases) and serological analyses (76.5 %, 65 positive results out of 85 cases). A lower percentage was observed for the urine antigen test (57.1 %, 48 positive results out of 84), but this increased to 75.8 % (47 positive results out of 62) for cases caused by L. pneumophila serogroup 1. The PCR-based methods gave always positive results in all 16 examined cases.

Hospital setting involved
Table 3 lists the bacteria responsible for the waterborne HCAIs according to the setting of the case occurrence. Intensive care units (ICUs) were the most frequently involved (41.6 %), whereas 24 articles (31.2 %) described cases in oncology/haematology, surgical transplant and dialysis/haemodialysis, and a variety of other wards accounted for 18.2 % of articles.

The settings were reported in 25 out of 48 articles (52.1 %) describing Legionella pneumonia and were intensive care (Aubert et al., 1990; Tram et al., 1990; Holmberg et al., 1993; Lück et al., 1994; Venezia et al., 1994; Bangsberg et al., 1995; Graman et al., 1997; Borau et al., 2000), surgical transplant unit (Benz-Lemoine et al., 1991; Levin et al., 1991; Campins et al., 2000; Trübel et al., 2002; Bou & Ramos, 2009; Cheng et al., 2012), oncology/haematology unit (Johansson et al., 2006; Gudijol et al., 2007; Brület et al., 2008; Palmore et al., 2009; Mencacci et al., 2011), rehabilitation ward (Nechwatel et al., 1993; Hoebe et al., 1998; Torri et al., 2003), nursery/delivery room (Franzin et al., 2001; Shachor-Meyouhas et al., 2010) and cardiology (Levy et al., 2003).
Table 1. Genera of bacteria responsible for waterborne HCAI

<table>
<thead>
<tr>
<th>Family and species</th>
<th>No. of articles</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Legionellaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. pneumophila serogroup 1+8</td>
<td>2</td>
<td>Aubert <em>et al</em>. (1990); Mermel <em>et al</em>. (1995)</td>
</tr>
<tr>
<td>L. micdadei</td>
<td>1</td>
<td>Knirsch <em>et al</em>. (2000)</td>
</tr>
<tr>
<td>L. pneumophila serogroup 1+6</td>
<td>1</td>
<td>Bangsborg <em>et al</em>. (1995)</td>
</tr>
<tr>
<td>L. pneumophila serogroup 1+12</td>
<td>1</td>
<td>Lück <em>et al</em>. (1998)</td>
</tr>
<tr>
<td><strong>Moraxellaceae</strong></td>
<td>4</td>
<td>Rees &amp; Allen (1996); Kappestein <em>et al</em>. (2000); Mittal <em>et al</em>. (2003); Hong <em>et al</em>. (2012)</td>
</tr>
<tr>
<td><strong>Sphingomonadaceae</strong></td>
<td>4</td>
<td>Perola <em>et al</em>. (2002b); Kilic <em>et al</em>. (2007); Meric <em>et al</em>. (2009); Mutlu <em>et al</em>. (2011)</td>
</tr>
<tr>
<td><strong>Flavobacteriaceae</strong></td>
<td>3</td>
<td>Pokrzyska <em>et al</em>. (1993); Hoque <em>et al</em>. (2001); Mosayebi <em>et al</em>. (2011)</td>
</tr>
<tr>
<td><strong>Campylobacteriaceae</strong></td>
<td>1</td>
<td>Rautelin <em>et al</em>. (1990)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>125</td>
<td></td>
</tr>
</tbody>
</table>

Source of infection

Drinking water was the most frequently reported source of infection. The contamination was mainly at the point of use, such as sink, shower and bathtub (Khardori *et al.*, 1990; Burns *et al.*, 1991; Bert *et al.*, 1998; Ferroni *et al.*, 1998; Verweij *et al.*, 1998; Berthelot *et al.*, 2001; Hoque *et al.*, 2001; Su *et al.*, 2001; Trautmann *et al.*, 2001; Vochem *et al.*, 2001; Sakhnini *et al.*, 2002; Mittal *et al.*, 2003; Blanc *et al.*, 2004; Horcajada *et al.*, 2006; Kilic *et al.*, 2007; Cooksey *et al.*, 2008; Breathnach *et al.*, 2012; Hong *et al.*, 2012; Lowe *et al.*, 2012). The colonization of taps was associated with scarce cleaning/disinfection procedures and was favoured by aerators (Weber *et al.*, 1999; Kappestein *et al.*, 2000; Perola *et al.*, 2002b) and automatic devices (Kotsanas *et al.*, 2008; Livni *et al.*, 2008; Durojaïe *et al.*, 2011; Yapicioglu *et al.*, 2012). Curiously, the tap-water drainage system was also involved in infections mainly sustained by Pseudomonadaceae (Gillespie *et al.*, 2000; Lucero *et al.*, 2011; Breathnach *et al.*, 2012; Schneider *et al.*, 2012). In other episodes, bacteria colonized the entire water distribution system (Rautelin *et al.*, 1990; Pegues *et al.*, 1994; Conger *et al.*, 2004; Kline *et al.*, 2004; Tobin-D’Angelo *et al.*, 2004; Aumeran *et al.*, 2007; Livni *et al.*, 2008), mainly when water chlorination was low or absent
Table 2. Methods performed to diagnose Legionella pneumonia

<table>
<thead>
<tr>
<th>Diagnostic method(s)</th>
<th>No. of articles</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture + one tool</td>
<td>12</td>
<td>Trübel et al. 2002; Bencini et al. 2005; Gudiol et al. 2007; Engelhart et al. 2008; Bou &amp; Ramos 2009; Chien et al. 2010</td>
</tr>
<tr>
<td>+ urine antigen</td>
<td>6</td>
<td>Holmberg et al. 1993; Venezia et al. 1994; Lück et al. 1998; Campins et al. 2000</td>
</tr>
<tr>
<td>+ serology</td>
<td>4</td>
<td>Shachor-Meyouhas et al. 2010</td>
</tr>
<tr>
<td>+ PCR</td>
<td>1</td>
<td>Lück et al. 1994</td>
</tr>
<tr>
<td>+ direct fluorescence</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Culture + two tools</td>
<td>13</td>
<td>Darelid et al. 1994; Patterson et al. 1994; Knirsch et al. 2000; Oren et al. 2002; Levy et al. 2003; Torri et al. 2003; Brület et al. 2008</td>
</tr>
<tr>
<td>+ urine antigen + serology</td>
<td>7</td>
<td>Aubert et al. 1990; Berthelot et al. 1998; Perola et al. 2002a</td>
</tr>
<tr>
<td>+ serology + direct fluorescence</td>
<td>3</td>
<td>Fendukly et al. 2007; Palmore et al. 2009; Mencacci et al. 2011</td>
</tr>
<tr>
<td>+ urine antigen + PCR</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Culture + three tools</td>
<td>4</td>
<td>Ozerol et al. 2006; Lai et al. 2010; Cheng et al. 2012</td>
</tr>
<tr>
<td>+ urine antigen + serology + direct fluorescence</td>
<td>1</td>
<td>Chang et al. 1996</td>
</tr>
</tbody>
</table>

(Rautelin et al., 1990; Pegues et al., 1994; Kline et al., 2004; Aumeran et al., 2007; Livni et al., 2008). Tap water was an indirect source of HCAI for contamination of equipment for haemodialysis (Lowry et al., 1990; Vanholder et al., 1990; Jackson et al., 1994; Magalhães et al., 2003; Souza et al., 2004) and hydrotherapy (Kolmos et al., 1993; Richard et al., 1994; De Vos et al., 1997; Mansour et al., 2008). Moreover, nosocomial waterborne infection occurred when tap water was used inappropriately to dilute detergent/disinfectant solutions (Rudnick et al., 1996; Nasser et al., 2004; Aumeran et al., 2007; Romero-Gómez et al., 2008), to administer drugs or food (Bert et al., 1998; Bukholm et al., 2002) and to wash medical equipment (Pazzaglia et al., 1990; Meyers et al., 2002; Randrianirina et al., 2009).

In six articles, distilled water was the source of infection due to its contamination during storage (Wang et al., 1991; Kendirli et al., 2004; Meric et al., 2009), production (Maroye et al., 2000; Mosayebi et al., 2011) and the reverse osmosis process in a haemodialysis system (Yan et al., 2008). Another three articles (Maki et al., 1991; Otağ et al., 2005; Yan et al., 2008) identified distilled water as a source of cases without finding the cause of contamination. Mutlu et al. (2011) described an outbreak of Sphingomonas paucimobilis in a neonatal ICU and hypothesized that the distilled water used for humidifying incubators and mechanical ventilators could have been the source of the outbreak, although the environmental isolates differed from the clinical ones. Sterile (Roberts et al., 1990; Lacey & Want, 1991; Douce et al., 2008) and bottled (Moreira et al., 2005; Eckmanns et al., 2008; Naze et al., 2010) water caused HCAIs when the original commercial products were contaminated. Burkholderiaceae were the most common contaminant for distilled (Maki et al., 1991; Maroye et al., 2000; Kendirli et al., 2004; Otağ et al., 2005; Yan et al., 2008) and sterile (Roberts et al., 1990; Lacey & Want, 1991; Douce et al., 2008) water, whilst bottled water was mainly colonized by Pseudomonas aeruginosa (Eckmanns et al., 2008; Naze et al., 2010).

Curious and unexpected sources of infection were water-containing toys (Buttery et al., 1998), a soap used for hand washing (Fanci et al., 2009), leeches infected in a contaminated aquarium (Sartor et al., 2002), an ice machine (Wang et al., 2009), fentanyl intentionally replaced with distilled water (Maki et al., 1991), a water bath used to thaw fresh frozen plasma and human albumin (Muyldermans et al., 1998), and condensate from ventilator and humidifier water traps (Gorman et al., 1993; Pokrywka et al., 1993; Jeong et al., 2001; Lee, 2008). Lastly, two articles described two HCAIs after exposure of patients to holy water: one was a burn patient infected by Acinetobacter baumanii (Rees & Allen, 1996) and the other was a multiply injured patient infected by P. aeruginosa (Greaves & Porter, 1992).

Table 4 shows the sources of Legionella and the prevention/control measures adopted after the case occurrence. The hot-water distribution system was the prevalent source of infection (68.7 %), followed by contaminated medical devices, which were mainly implicated before 2001 (14.6 %). Unusual sources of Legionella infection were ice machines and decorative fountains. A number of control measures were adopted according to the source of infection and building and/or patient characteristics.
Table 3. Genera responsible for waterborne HCAIs according to the hospital setting

Results are shown as the number of articles and the references.

<table>
<thead>
<tr>
<th>Family</th>
<th>Intensive care</th>
<th>Oncology/haematology</th>
<th>Surgical transplant</th>
<th>Dialysis/haemodialysis</th>
<th>Other*</th>
<th>Not reported</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonadaceae</em></td>
<td>10: Bert et al. (1998); Muyldermons et al. (1998); Berthelot et al. (2001); Trautmann et al. (2001); Bukholm et al. (2002); Blanc et al. (2004); Eckmanns et al. (2008); Naze et al. (2010); Durojaie et al. (2011); Yapicioglu et al. (2012)</td>
<td>5: Buttery et al. (1998); Gillespie et al. (2000); Aumeran et al. (2007); Fanci et al. (2009); Schneider et al. (2012)</td>
<td>1: Ferroni et al. (1998)</td>
<td>1: Vanholder et al. (1990)</td>
<td>6: Greaves &amp; Porter (1992); Kolmos et al. (1993); Richard et al. (1994); De Vos et al. (1997); Vochem et al. (2001); Mansour et al. (2008)</td>
<td>1: Breathnach et al. (2012)</td>
</tr>
<tr>
<td><em>Burkholderiaceae</em></td>
<td>5: Kendirli et al. (2004); Otağ et al. (2005); Douce et al. (2008); Lee (2008); Lucero et al. (2011)</td>
<td>1: Lacey &amp; Want (1991)</td>
<td>1: Maki et al. (1991)</td>
<td>4: Magalhães et al. (2003); Souza et al. (2004); Romero-Gómez et al. (2008); Yan et al. (2008)</td>
<td>2: Maroye et al. (2000); Kotsanas et al. (2008)</td>
<td>3: Roberts et al. (1990); Nasser et al. (2004); Moreira et al. (2005)</td>
</tr>
<tr>
<td><em>Mycobacteriaceae</em></td>
<td>0</td>
<td>3: Kline et al. (2004); Cooksey et al. (2008); Livni et al. (2008)</td>
<td>1: Meyers et al. (2002)</td>
<td>1: Lowry et al. (1990)</td>
<td>1: Burns et al. (1991)</td>
<td>3: Conger et al. (2004); Tobin-D’Angelo et al. (2004); Wang et al. (2009)</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>6: Wang et al. (1991); Gorman et al. (1993); Jeong et al. (2001); Su et al. (2001); Horcajarada et al. (2006); Lowe et al. (2012)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1: Randrianirina et al. (2009)</td>
<td>0</td>
</tr>
<tr>
<td><em>Moraxellaceae</em></td>
<td>2: Mittal et al. (2003); Hong et al. (2012)</td>
<td>1: Kappstein et al. (2000)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1: Rees &amp; Allen (1996)</td>
</tr>
<tr>
<td><em>Sphingomonadaceae</em></td>
<td>2: Meric et al. (2009); Mutlu et al. (2011)</td>
<td>2: Perola et al. (2002b); Kilic et al. (2007)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Xanthomonadaceae</em></td>
<td>3: Khardori et al. (1990); Verweij et al. (1998); Weber et al. (1999)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1: Sakhnini et al. (2002)</td>
<td>0</td>
</tr>
<tr>
<td><em>Flavobacteriaceae</em></td>
<td>3: Pokrywka et al. (1993); Hoque et al. (2001); Mosayebi et al. (2011)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Aeromonadaceae</em></td>
<td>0</td>
<td>0</td>
<td>1: Sartor et al. (2002)</td>
<td>0</td>
<td>1: Pazzaglia et al. (1990)</td>
<td>0</td>
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<tr>
<td><em>Campylobacteraceae</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1: Rautelin et al. (1990)</td>
<td>0</td>
</tr>
<tr>
<td>Gram-negative</td>
<td>1: Pegues et al. (1994)</td>
<td>0</td>
<td>1: Rudnick et al. (1996)</td>
<td>1: Jackson et al. (1994)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>32</strong></td>
<td><strong>12</strong></td>
<td><strong>5</strong></td>
<td><strong>7</strong></td>
<td><strong>14</strong></td>
<td><strong>7</strong></td>
</tr>
</tbody>
</table>

*Burn unit, urology ward, rehabilitation ward, nursery, delivery ward, cardiology.
Table 4. Sources of infection and prevention/control measures adopted in Legionella HCAIs

<table>
<thead>
<tr>
<th>Source of infection (no. of articles)</th>
<th>References</th>
<th>Preventive and control measures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water distribution system (34)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot water (29)</td>
<td>Aubert et al. (1990); Tram et al. (1990); Benz-Lemoine et al. (1991); Levin et al. (1991); Struelens et al. (1992); Blatt et al. (1993); Holmberg et al. (1993); Nechwatal et al. (1993); Darelid et al. (1994); Mermel et al. (1995); Chang et al. (1996); Green et al. (1996); Berthelot et al. (1998); Lück et al. (1998); Jonas et al. (2000); Knirsch et al. (2000); Franzin et al. (2001); Oren et al. (2002); Perola et al. (2002a); Trübel et al. (2002); Torii et al. (2003); Tercieli-Zorman et al. (2004); Ozerol et al. (2006); Gudiol et al. (2007); Fendukly et al. (2007); Chien et al. (2010); Shachor-Meyouhas et al. (2010); Menacchi et al. (2011); Cheng et al. (2012)</td>
<td>Shock treatment: heating and flushing, shock chlorination; continuous treatments: chlorine, copper–silver ions, UV light; installation of point-of-use filter; outlets disinfection; removal of showers with hose or head handle; water network renovation; hot-water temperature: &gt;50 °C; cold-water temperature: &lt;20 °C.</td>
</tr>
<tr>
<td>Hot + cold water (4)</td>
<td>Patterson et al. (1994); Marrie et al. (1995); Johansson et al. (2006); Bruel et al. (2008)</td>
<td>Sterile water for filling, rinsing or cleaning, sodium hypochlorite disinfection, disposable instrument</td>
</tr>
<tr>
<td>Cold water (1)</td>
<td>Hoebe et al. (1998)</td>
<td>Shock chlorination</td>
</tr>
<tr>
<td><strong>Devices (7)</strong></td>
<td></td>
<td>Mechanical cleansing, removal</td>
</tr>
<tr>
<td><strong>Ice machine (3)</strong></td>
<td>Mastro et al. (1991); Lück et al. (1994); Venezia et al. (1994); Campins et al. (2000); Borau et al. (2000); Levy et al. (2003); Bou &amp; Ramos (2009)</td>
<td>Mechanical cleansing + shock chlorination, replace direct by indirect cooling tower</td>
</tr>
<tr>
<td><strong>Decorative fountain (2)</strong></td>
<td>Bangsborg et al. (1995); Graman et al. (1997); Bencini et al. (2005)</td>
<td>Mechanical cleansing, removal</td>
</tr>
<tr>
<td><strong>Cooling tower (1)</strong></td>
<td>Engelhart et al. (2008)</td>
<td>Mechanical cleansing, removal</td>
</tr>
<tr>
<td><strong>Not reported</strong> (1)</td>
<td>Lai et al. (2010)</td>
<td>Mechanical cleansing, removal</td>
</tr>
</tbody>
</table>

*B Medication nebulizer, oxygen nebulizer, oxygen humidifier, incubator humidifier, nasogastric tube, transoesophageal echocardiography probe.

Bacterial typing to determine associations between environmental and clinical isolates

Two distinct typing systems were described to match strains isolated from clinical specimens and environmental source. Phenotyping methods included antibiotic susceptibility (Rees & Allen, 1996; Maroye et al., 2000; Jeong et al., 2001; Su et al., 2001; Mittal et al., 2003; Kendirli et al., 2004; Otağ et al., 2005; Aumeran et al., 2007; Kilic et al., 2007; Randrianirina et al., 2009; Schneider et al., 2012), serology (Rautelin et al., 1990; Kolmos et al., 1993; Trautmann et al., 2001), biochemical tests (Rees & Allen, 1996; Maroye et al., 2000), phage typing (Greaves & Porter, 1992; Kolmos et al., 1993) and whole-cell protein electrophoresis (Magalhães et al., 2003). Among the genotyping techniques, PFGE was prevalent (Burns et al., 1991; Bert et al., 1998; Buttery et al., 1998; Ferroni et al., 1998; Weber et al., 1999; Gillespie et al., 2000; Berthelot et al., 2001; Hoque et al., 2001; Jeong et al., 2001; Su et al., 2001; Vochem et al., 2001; Meyers et al., 2002; Magalhães et al., 2003; Blanc et al., 2004; Conger et al., 2004; Tobin-D’Angelo et al., 2004; Moreira et al., 2005; Horcajada et al., 2006; Kilic et al., 2007; Cooksey et al., 2008; Douce et al., 2008; Eckmanns et al., 2008; Mansour et al., 2008; Romero-Gómez et al., 2008; Yan et al., 2008; Méric et al., 2009; Randrianirina et al., 2009; Wang et al., 2009; Lucero et al., 2011; Mutlu et al., 2011; Lowe et al., 2012; Yapicioglu et al., 2012), followed by randomly amplified polymorphic DNA (De Vos et al., 1997; Myllymers et al., 1998; Verweij et al., 1998; Kappstein et al., 2000; Maroye et al., 2000; Perola et al., 2002b; Kline et al., 2004; Souza et al., 2004; Otağ et al., 2005; Cooksey et al., 2008; Schneider et al., 2012). Other genotypic methods were RFLP (Magalhães et al., 2003; Nasser et al., 2004; Kotsanas et al., 2008), amplified fragment length polymorphism (Fanci et al., 2009; Naze et al., 2010), arbitrarily primed PCR (Trautmann et al., 2001), repetitive-element PCR (Cooksey et al., 2008; Kotsanas et al., 2008), variable-number tandem repeat (Naze et al., 2010; Durojaiye et al., 2011), isoelectric focusing of pyoverdine (De Vos et al., 1997), infrequent restriction site PCR (Su et al., 2001), multilocus enzyme electrophoresis (Kline et al., 2004), enterobacterial repetitive intergenic consensus PCR (Aumeran et al., 2007), PCR restriction analysis (Cooksey et al., 2008), Sau-PCR (Yan et al., 2008), multilocus sequence typing (Hong et al., 2012), microarray (Schneider et al., 2012), plasmid profile analysis (Wang et al., 1991) and ribotyping (Souza et al., 2004).
2008; Palmore et al., 2009) and amplified fragment length polymorphism (Campins et al., 2000; Jonas et al., 2000; Perola et al., 2002a; Bencini et al., 2003; Johansson et al., 2006; Fendukly et al., 2007; Mencacci et al., 2011). Other DNA fragment-based methods such as randomly amplified polymorphic DNA (Hoebe et al., 1998; Trübel et al., 2002; Perola et al., 2002a), restriction enzyme analysis (Lück et al., 1994; Bangsberg et al., 1995; Jonas et al., 2000), arbitrarily primed PCR (Berthelot et al., 1998; Jonas et al., 2000) and RFLP (Darelid et al., 1994) were less frequently reported. The ribotyping method was performed in two investigations occurring before 2000 (Bangsberg et al., 1995; Berthelot et al., 1998), whereas sequence-based typing, a DNA sequencing method, was described in two recent articles (Fendukly et al., 2007; Engelhart et al., 2008). Lastly, mAb typing was used in seven reports (Aubert et al., 1990; Struelens et al., 1992; Nechwatal et al., 1993; Blatt et al., 1993; Darelid et al., 1994; Berthelot et al., 1998; Engelhart et al., 2008), but in two of them was not useful to relate patients to environmental isolates and thus genotypic techniques were applied to identify the source of the outbreak (Struelens et al., 1992; Nechwatal et al., 1993).

**Discussion**

Numerous procedures and activities, regularly conducted during the care of patients admitted to healthcare facilities, require water and/or water solutions. By reviewing 22 years of international literature, we documented a substantially unchanged frequency of waterborne HCAIs, all caused by opportunistic bacteria. In total, 48 articles describing *Legionella* infections and 77 involving other bacteria were published, suggesting that they represent the tip of an iceberg. A possible explanation for this limited number of published articles is that water is no longer considered a hygiene problem in industrialized countries, whereas other care priorities prevail in disadvantaged countries, justifying the relatively low number of detailed studies conducted on water as a source of HCAIs.

*Legionella* was the leading cause of waterborne HCAIs among patients, and *L. pneumophila* serogroup 1 was the predominant organism causing hospital outbreaks (66.7% of outbreaks), followed by serogroups 6 and 3, whereas a *Legionella* non-*pneumophila* case was described in only one article (Knirsch et al., 2000). The number of nosocomial clusters/outbreaks of legionnaires’ disease decreased in the last 10 years, most probably due to the implementation of diagnostic tests that allowed the early detection of cases and the rapid adoption of appropriate control measures, thus preventing the occurrence of additional cases (Marchesi et al., 2011). As it is not possible to distinguish patients with legionnaires’ disease from other forms of pneumonia by clinical or radiological means, laboratory confirmation is essential for diagnosis (Blyth et al., 2009; Bargellini et al., 2013). Isolation of *Legionella* spp. by culture is still considered the ‘gold standard’ for diagnosis (Jarraud et al., 2013), as confirmed by our review. The use of direct fluorescence techniques is limited as they require experienced laboratory personnel; furthermore, false-positive results may occur, so a positive fluorescence result in the absence of other supporting evidence is generally not accepted as sufficient for the diagnosis of *Legionella* infection (Murdoch, 2003). Serology is not a useful diagnostic tool in the early stage of disease, but our review showed that it has constantly been used over the investigated years to confirm the disease (seroconversion) and/or to document past infection for epidemiological investigation after outbreaks (Diederan, 2008). Nowadays, the urinary antigen test is the most common diagnostic technique for *Legionella* infection (Helbig et al., 2012), but a significant proportion of false-negative results were observed in waterborne HCAIs (42.8%). This is in line with the presence of pneumonia caused by legionellae other than *L. pneumophila* serogroup 1 that are not easily detectable with commercial urinary tests (Tronel & Hartemann, 2009). PCR-based assays are increasingly attractive tools for the detection of legionellae in clinical samples, as they are able to detect all *Legionella* spp. and to provide rapid results (Diederan, 2008). A drawback of PCR, as with the other discussed diagnostic tests, is that it does not allow isolation of the organism; as a consequence, the need for a clinical isolate to trace the infection source still makes culture mandatory. Given the limitations of each diagnostic method, a combination of tests is always warranted.

Besides legionellae, we noted that *Pseudomonaceae* (mainly *P. aeruginosa*) and *Burkholderiaceae* (mainly *Pseudomonas pickettii* and *Burkholderia cepacia*) were the most frequent bacteria involved in waterborne HCAIs. The increase in antimicrobial-resistant pathogens, invasive procedures and immunocompromised and/or ageing patients recovering in hospital may explain the increase in number of cases in the last decade also involving *Mycobacteriaceae* and *Enterobacteriaceae*.

The severity of the patient’s illness was the main risk factor, and thus health workers, in particular nursing staff, should adopt any preventive measure necessary to avoid water exposure of immunosuppressed patients, and also when they move from the ward for diagnostic and/or care purposes. Furthermore, special attention should be devoted to children, who are rarely affected by waterborne infections of community origin. We found nine articles where hospitalized neonates, in some cases premature and/or receiving corticosteroids, got *Legionella* pneumonia directly from water or from mechanical ventilation (Aubert et al., 1990; Holmberg et al., 1993; Lück et al., 1994; Green et al., 1996; Campins et al., 2000; Franzin et al., 2001; Trübel et al., 2002; Johansson et al., 2006; Shachor-Meyouhas et al., 2010). Another 32 articles where neonates or children were affected by other waterborne bacteria are described (Pazzaglia et al., 1990; Lacey & Want, 1991; Wang et al., 1991; Pegues et al., 1994; Buttery et al., 1998; Ferroni et al., 1998; Muyldermans et al., 1998; Verweij et al., 1998; Kappstein et al., 2000; Maroye et al., 2000; Hoque et al., 2001; Jeong et al., 2001; Su et al., 2001; Vochem et al., 2001;
Perola et al., 2002b; Mittal et al., 2003; Kendirli et al., 2004; Kline et al., 2004; Moreira et al., 2005; Aumeran et al., 2007; Kilic et al., 2007; Lee, 2008; Kotsanas et al., 2008; Livni et al., 2008; Randrianirina et al., 2009; Naze et al., 2010; Lucero et al., 2011; Mosayebi et al., 2011; Mutlu et al., 2011; Hong et al., 2012; Schneider et al., 2012; Yapicioglu et al., 2012).

Drinking-water and hot-water distribution systems are the primary reservoir of bacteria responsible for waterborne HCAIs occurring through contact, inhalation and ingestion (Anaissie et al., 2002; Exner et al., 2005; Cervia et al., 2008). By reviewing the literature data, unusual sources of infection such as water-retaining toys, leeches infected in a contaminated aquarium and holy water have emerged, illustrating the wide variety of water uses during care of patients.

Even with a thorough investigation, it can be difficult to track down the index strain from the environmental source of infection. To provide useful indications to clinicians and clinical microbiologists on how to proceed when a case occurs, we suggest obtaining clinical samples from all patients admitted to the ward where the first case appeared; this will allow establishment of the extent of the infection. We recommend planning and carrying out environmental investigations as soon as possible in order to increase the chances of identifying the common source of the outbreak. For this purpose, every possible occasion of water and/or other liquid exposure should be checked, and swabs and/or water samples should be collected according to the patient’s movements in the hospital. Interviews with hospital staff should be carried out to gather information concerning possible incorrect practices, such as the use of a catheter that is not properly covered during bathing or the dilution of a disinfectant with tap water.

In order to correlate clinical and environmental strains, phenotyping methods such as antibiotic susceptibility, serotyping, biochemical tests and phage typing were mainly used in older studies (Rautelin et al., 1990; Greaves & Porter, 1992; Kolmos et al., 1993; Rees & Allen, 1996; Mittal et al., 2003; Kendirli et al., 2004). As expected, these methods appeared to be weakly discriminatory in distinguishing closely related strains. mAb subgrouping, another phenotyping method, limited to L. pneumophila, has a low discriminatory index but is cheap and easy to perform, and is a useful screening tool in outbreak investigations when many strains have to be characterized (Lück et al., 2013). Molecular typing techniques were performed in more recent studies where the usefulness of such methods for the certain identification of the environmental source of the outbreak was confirmed. DNA banding pattern-based methods were the most frequently applied genotyping techniques. Among these, PFGE is considered the ‘gold standard’ for subtyping many bacteria. As this method has the advantage of being used in several countries, many web resources for bacterial genotyping exist; however, it is time- and labour-consuming and it lacks reproducibility and inter-laboratory comparability. Many other techniques are available, such as DNA hybridization-based (microarrays) and DNA sequencing-based methods (multilocus sequence typing and sequence-based typing). Each has advantages and limitations that make them useful in some studies and restrictive in others. The choice will depend on the available skill level, the resources of the laboratory and the study purpose (Ranjbar et al., 2014). We wish to stress that many techniques should be combined in order to increase the discriminatory power of the whole test battery.

Preventive and control measures

A key element for the reduction of environmental microbial contamination and the protection of high-risk patients is the implementation of adequate control strategies. Those that have proved effective and those with some level of evidence for effectiveness in reducing rates of waterborne HCAIs are reported in published guidelines and various studies (Anaissie et al., 2002; Exner et al., 2005; Freije, 2005; Sheffer et al., 2005; Curtis, 2008; Marchesi et al., 2013). Water disinfection is generally insufficient to control the risk of infection, so a complex prevention plan should be put in place (WHO, 2008; Decker & Palmore, 2013).

The most relevant and simple measures aimed at reducing the risk of waterborne HCAIs should include: (i) the education of all direct care providers and family members to minimize patient exposure to tap water; (ii) the provision of sterile water to immunocompromised patients; (iii) paying attention to other less common water uses and to devices requiring water; (iv) organizing a programme of periodic cleaning and maintenance of showers, baths and sinks; (v) installing disinfection systems and/or point-of-use filters on taps and shower heads in those settings where patients at high risk of opportunistic infections are admitted; and (vi) avoiding the installation of other potential sources of infection such as decorative pools and fountains.

National and international guidelines for the prevention of legionnaires’ disease have been published, but definitive and standardized solutions are not yet available. In our opinion, those responsible for controlling environmental Legionella contamination and for taking precautions for the prevention of legionnaires’ disease should follow these recommendations: (i) form a team of all interested professionals: engineers, technicians, nurses, clinicians, microbiologists and public health doctors; (ii) examine the environment where the micro-organism can be found, and identify the critical points in the water distribution system; (iii) estimate the risk in terms of number of exposed persons, health status of patients and virulence of isolated legionellae; and (iv) take decisions after a careful cost–benefit analysis.

Conclusion

The revision of published articles on water-associated HCAIs highlights the fact that a single standardized model
for the case management and adoption of preventive/control measures does not currently exist; all the suggested strategies have to be revised and adapted case by case.

As bacterial water contamination appears to be unavoidable, we strongly advise nursing staff to pay special attention to children and immunosuppressed patients in terms of tap-water exposure and also their personal hygiene, and to regularly use sterile water for rinsing/cleaning devices.

Lastly, clinicians, nurses, microbiologists, hygienists and technicians should implement the current knowledge on waterborne HCAIs and construct an integrated network of activities aimed at preventing and controlling exposure to contaminated water, thus reducing the occurrence of related diseases, with indisputable advantages for the health of patients and their families.

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