

Are liquids an efficient vehicle of healthcare associated infections? A review of reported cases in Italy (2000-2014)

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Abstract

Introduction. In the field of healthcare-associated infections (HCAIs), one of the most reported, studied and discussed sources of infections is water, partly due to its controllability, but also because healthcare facilities, especially hospitals, require a significant quantity of water per day. In addition to water, during healthcare procedures, other liquids can serve as source of infections. The present study reports a review of those HCAIs associated to liquid vehicles occurred in Italy during the period 2000-2014.

Method. The review focused on cases of liquid-associated HCAIs in both sporadic cases and outbreaks according to the definition provided by both World Health Organization and United States' Centers for Disease Control and Preventions in 2011. The review included all original papers published in peer-reviewed journals, in which the association between the infection and the exposure to contaminated water/other fluid was demonstrated by epidemiological and/or molecular methods. Articles describing cases due to parenteral transmitted pathogens (by blood or blood-derived fluids) were excluded.

Results. During the period 2000-2014, 34 episodes have been described for a total of about 400 cases of infection. Isolations included genus *Legionella*, *Pseudomonas*, *Serratia*, *Ralstonia*, *Burkholderia*, *Klebsiella* and other pseudomonadaceae. The results confirm that HCAIs can be associated also to liquids other than piped water. The large majority of articles refers to hospital wards where patients with high risk of infections are usually admitted.

Discussion. The review highlights a great number of HCAIs, but if we consider that the large part of HCAIs are not reported in literature, it is clear that the burden of this phenomenon is by far higher. Many cases of HCAI were identified in the context of local surveillance systems, demonstrating their role in HCAI control. With regard to diagnosis, the isolation and identification of the etiological agent is critical to reach the source of infection and to plan the necessary disinfection measures. Therefore, it is possible to conclude that, through a multiple approach of engineering and hygiene measures, as well as surveillance and management of hospital liquids, the risk for contracting "water born" HCAIs may be controlled.

Introduction

Traditionally, infections were classified as "community-" or "hospital-acquired", according to their place of acquisition; but,

over the last decades, the increasing weight of outpatient clinical care has led to the new comprehensive definition of "healthcare-associated infections" (HCAIs) (1) opposed to the "community-acquired infections".

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Actually the complexity of modern healthcare facilities and the increasing proportion of immunologically compromised patients make prevention of HCAs a high priority (2).

Several clinical studies report data on HCAs using one of their first definition by Friedman et al. (3), according to which HCAs are infections present at hospital admission or within 48 hours of admission in patients that fulfilled any of the following criteria: received intravenous therapy at home, wound care or specialized nursing care in the previous 30 days; attended a hospital or haemodialysis clinic or received intravenous chemotherapy in the previous 30 days; were hospitalized in an acute care hospital for ≥ 2 days in the previous 90 days, resided in a nursing home or long-term care facility (3). More easily, today, HCAs are considered as infections that patients acquire while receiving treatment for medical or surgical conditions (4).

Worldwide, HCAs are the cause of significant morbidity, mortality, and financial burden; moreover, the use of antibiotics to treat these infections leads to increased microbial resistance (4-6). Nevertheless, the true burden of HCAs is still unknown in many places, particularly in developing countries (4).

In Italy, there are no surveillance systems that routinely provide estimates of the national burden of such infections. One recent review produced by the National Institute of Health (ISS) reports 450,000-700,000 HCAs per year, 30% of which is considered preventable. Even more difficult is to stratify HCAs according to the source of infection (7).

Among the numberless HCAs sources of infections, one of the most reported, studied and discussed is water, in part due to its controllability (8), but also because healthcare facilities require a significant amount of water per day, to be used in different ways and for different

purposes (9). Several factors have a great impact on the amount of water used; these include: bed number, number and type of wards and units, building age, access to water, general services present inside the structure, institutional management policies and awareness in managing the structure in order to safeguard the environment, climate, cultural and geographical factors. Therefore, the amount of water used in healthcare facilities varies from 200 to 1200 L/bed/day (10). Disinfecting the drinking water system is an effective preventive measure; even though, by itself, disinfection is not enough for eliminating the risk of HCAs, since no treatment can sterilize forever the water system. Therefore, the efficacy of any disinfection measures should be correctly chosen and validated (11), taking into account the different factors that contribute to the persistent contamination of the water systems (eg. large and complex water systems with areas of low flow that predispose to stagnation and biofilm formation, the presence of dead legs, amoebae, the variable microbial load of contamination, water temperatures that are ideal for bacterial growth, etc) (2,12-14).

In addition to water, during healthcare procedures other liquids can serve as source of infections. Soap solutions, disinfectants, saline and other liquid vehicles, when contaminated at the origin or not well stored and managed, can cause HCAI both in sporadic and epidemic forms (15); and this is particularly true in those wards admitting high risk patients.

Since continued improvements in patient safety depend on maintaining a comprehensive understanding of the epidemiology of HCAs (16), the present study reports a review of HCAs caused by liquid vehicles which occurred in Italy during the period 2000-2014, and were described in a scientific publication.

Methods

The review focused on single cases of liquid-related infections (LRIs) and liquid-related outbreaks (LROs), occurred in Italy from January 1, 2000 to December 31, 2014 and present in the data banks by June 30, 2016. In order to obtain a more reliable coverage of all published reports of these years, a *literature search* using *Web of Science* and *PubMed* was performed. *Outbreak database* (<http://www.outbreak-database.com>) was also analyzed. The search included the bacterial name plus “waterborne” and one or more of the following terms: “outbreak”, “case report”, “infection”, “nosocomial” and “Italy”. The pathogen name was also associated with the setting of appearance, such as “intensive care”, “dialysis”, “onco-haematological”. Finally, the search included “water”, “soap”, “fluid”, “solution”, “disinfectant” plus “outbreak”, “case report” and “hospital” or “healthcare” and “Italy”.

The review included all original papers published in peer-reviewed journals, in English or in Italian, in which the association between infection and exposure to contaminated water/other fluids was demonstrated with epidemiological or molecular methods. Furthermore, we excluded all articles describing outbreaks or cases due to fecal-oral transmission, parenterally transmitted pathogens via blood or blood-derived fluids. Pseudo-infections and pseudo-outbreaks were also excluded. Duplicate references were also excluded. *Case definition* followed (World Health Organization 2011 (4) and United States' Centers Disease Control and Prevention 2011 indications (17)).

The articles were analyzed for the following information: pathogens involved, source, method(s) used to link patient and environmental isolates, setting. All reported cases/outbreaks were classified in Class I-II according to the *strength of evidence*

in terms of (a) epidemiologic and clinical laboratory data and (b) environmental data implicating water as the vehicle of transmission (17). According to a modified version of CDC classification (17): class I is assigned when both (a) and (b) criteria are fulfilled and adequate molecular data link at least one person to the implicated water or other fluid; class II is assigned when one of the two criteria (a or b) is not fulfilled or inadequate, but the scientific evidences suggest a link between at least one case of HCAI and an environmental exposure. All the studies in which the link between liquids and infections was neither proved nor suspected on the basis of scientific evidences were excluded.

Case count provided in this review refers only to the number of cases for which sufficient supporting evidence of correlation among water/other liquid vehicles and infection was provided in each paper. All selected papers have been analyzed to obtain:

- cases and deaths;
- isolations and susceptibility of organism;
- year of occurrence;
- infection sites;
- liquid vehicles of infections;
- settings (hospital wards or other healthcare structures);
- methods used to link vehicles with the infections.

The results have been described in two tables: the first table shows data regarding *Legionella* spp., the only agent included in a specific national surveillance system (NSS), among those investigated; the second refers to the others microorganisms (Tab. 1-2).

Results

From January 1, 2000 to June 30, 2016, 607 publications were identified as the result of the review and were reduced to 449 after

Table 1 - Cases and outbreaks of HCAI related to liquid vehicles due to Legionella spp - year 2000-2014

Source	Serogroups /Types	Years	Cases	Deaths	Reservoir	Setting (Wards - Units)	Method(s) used to link strains	Evidence
Franzin, 2004	sg. 1	2000	1	0	Water birth	Gynecology surgery Unit	Indirect immunfl., microagglutination, PCR	Class II
Boccia, 2006	sg. 1	2001 - 2004	1	0	Taps, showers water	Surgical, neurotraumatology Unit	Urinary antigens, PFGE	Class II
Montagna, 2006	sg. 1, sg. 2-14	2001-2005	12	0	Taps, showers	Internal Medicine Units	PFGE	Class II
Napoli, 2010	sg. 2-14, sg. 1, L. spp.	2000- 2009	9	n.r.	Taps, showers, others	Internal Medicine	PFGE	Class I
Ditomaso, 2006	sg. 1	2000-2002	9	n.r.	Tap water	Transplantation, Haematology, Oncology	RT-PCR	Class I
Triassi, 2006	sg1 /Philadelphia	2000-2003	2	n.r.	Drinking water	Cardiac Surgery Unit, Intensive Care Unit	PFGE	Class I
Ditomaso, 2007	sg. 3	2001-2002	1	n.r.	Drinking water	Hospital	Urinary antigen, col-ture	Class II
Galli, 2008, Bianchi, 2009	sg. 1	2003-2006	3	1	Showers water	Neurology, Nephrology, Internal Medicine Units	PGFE, AFLP, SBT	Class I
Leoni, 2006	sg. 1, sg. 5	2003	1	0	Hydrotherapy	SPA	Urinary antigens - serology, Immunofl.	Class II
Scaturro, 2007	sg1 /Philadelphia	2004	8	2	Drinking water, cooling towers	Hospital	AFLP	Class I
Ditomaso, 2014	sg. 1 subtype MAb 3/1, sg. 1, sg. 2-14	2004-2009	87	n.r.	Drinking water	Hospital	Urinary antige, Indirect immunfl.	Class II
Mencacci, 2011	sg. 3	2010	1	n.r.	Sinks, showers water	Oncology, Haematology	AFLP	Class I
Scaturro, 2011	sg.1 subtype Mab Olda	2010	1	1	Hospital water system	Pneumology Units	RT-PCR, nested SBT, AFLP	Class I
Ricci, 2012	sg. 1 ST 593	2011	1	1	Waterline	Dental Unit	MAT, SBT, AFLP	Class I

Class I = good evidence (a-epidemiologic and b-clinical laboratory data and c-environmental data available);

Class II = weak evidence (one of more criteria (a,b,c) are not provided or inadequate)

PFGE = Pulsed-field gel electrophoresis; **AFLP** = Amplified fragment length polymorphism; **RT-PCR** = Ribotyping polymerase chain reaction; **SBT** = Sequence-based typing; **MAT** = Monoclonal antibody typing; **PCR** = Polymerase Chain Reaction; **ST** = sequence type

Table 2 - Cases and outbreaks of HCAI related to liquid vehicles due to microbial agents other than *Legionella* spp. - year 2000-2014

Etiological agents	Source	Serogroups/ Types	Years	illnesses	Cases	Deaths	Reservoir	Setting (Wards - Units)	Method(s) used to link strains	Susceptibility of organism**	Evidence
<i>P. aeruginosa</i>	Fanci, 2006	Type A, B, C	2002	Bloodstream Infection	6	0	Showers water	Bone Marrow Transplantation Unit	AFLP	n.r.	class I
	Prospero, 2006		2004	Bloodstream Infection	4	0	Heparinized saline solutions	Internal Medicine Unit	API Bio Merieux, PFGE	Susceptible	class I
	Orsi, 2006	Pattern A,B^,C^	2005	Bloodstream Infection	9	1	Plasma-expander *	Neurosurgical Intensive Care Unit	PFGE	Susceptible	class I
	Crivaro, 2009	Type A, G	2005-2007	Bloodstream Infection	11	4	Sinks water, nurse hands	Neonatal Intensive Care Unit	PFGE	Resistant	class I
	Fanci, 2009		2006	Bloodstream Infection	6	0	Tap swab and soap dispenser	Stem Cells Transplant Unit	AFLP	Susceptible	class I
-	Lanini, 2011	ST 175	2007	Septicemia, pneumonia, UTI, vomit/diarrhoea	18	4	Soap dispenser *	Haematology Unit	RAPD, PFGE, AFLP MLST	Resistant	class I
<i>S. marcescens</i>	Pan, 2006		2004	Sepsis	6	1***	Total parenteral nutrition (TPN) solution	Surgery Unit	n.r.	Resistant	Class II
	Ligozzi, 2010	Type A	2007	Bacteremia, conjunctivitis, UTI, wound infection	6	0	Brest milk *	Neonatal Intensive Care Unit	Rep-PCR, PFGE	Resistant	class I
	Polilli, 2011		2011	Sepsis	5	2	Soap dispenser	Neonatal Intensive Care Unit	n.r.	n.r.	Class II
	Casolari, 2013	genotypes A-G	2003-2012	Sepsi, pneumonia	46	3	Soap dispenser and respiratory equipment	Neonatal Intensive Care Unit	RFLP-PCR	Susceptible/resistant clones	Class II
<i>R. pickettii</i> (Rp)	Pastici, 2005	type A (R1-R2), type B (R3)	2001	Systemic infection	4	0	Parenteral nutrition	Bone Marrow Transplant, Internal Medicine	PFGE, RAPD	Resistant	class I
	Pastici, 2005	type A (R1-R2)	2002	Systemic inflammatory response syndrome	9	2	Heparin solution	Oncology Unit	PFGE, RAPD	Resistant	class I
	D'Amico, 2005		2001-2002	Sepsis	28	0	Liquid solution for dialysis	Haemodialysis Unit	n.r.	Susceptible	Class II
<i>B. cenocepacia</i>	Lo Cascio, 2006,	Genomovar III B	2004-2005	Bloodstream Infection	38	0	Napkin disinfection *	Haemodialysis (Nephrology)	RFLP-PCR, PFGE	Susceptible	class I
<i>B. cepacea</i> complex	Righi, 2013		n.r.	Sepsis	13	6	Mouthwash	Intensive Care Unit	API Bio Merieux, RAPD	Resistant	class I
<i>K.pneumoniae</i>	Bagattini, 2006	Type B and C	2002-2004	Sepsis, meningitis, arthritis, pneumonia, ocular infections, UTI	19	n.r.	Baby incubators, sinks, other surfaces	Neonatal Intensive Care Unit	PFGE	Resistant	class I
<i>K.pneumoniae</i>	Fabbri, 2013		n.r.	Sepsis	6	0	Saccharose solution	Neonatal Intensive Care Unit	PFGE, Rep-PCR, AP-PCR, RAPD-PCR	Resistant	class I
<i>A.baumannii</i>	Longo, 2007		2005	VAP, meningitis, bloodstream Infection, wound infection,	14	4***	Washbasin, gloves, other surfaces	Intensive Care Unit	PFGE	Resistant	class I
<i>B. vesicularis</i>	Mondello, 2006		n.r.	Meningitis	1	0	Water taps	Neurosurgical division	API Bio Merieux	Susceptible	class II

* Water resulted contaminated with the same agent, but genetically different; **Resistant means resistant to 2 or more classes of antibiotics; ***Deaths were not directly attributable to the infection

^ Not responsible of the outbreak; § This number included both infections and colonizations; UTI = Urinary tract infection **PFGE** = Pulsed-field gel electrophoresis; **AFLP** = Amplified fragment length polymorphism; **RAPD** = Random amplified polymorphic DNA; **Rep-PCR** = Repetitive Extragenic palindromic PCR; **AP-PCR** = Arbitrary primed PCR; **MLST** = Multilocus Sequence Typing; **RFLP-PCR** = Restriction Fragment Length Polymorphism - Polymerase Chain Reaction; ARI = Acute respiratory illness; n.r. = Not reported; VAP = Ventilator Associated Pneumonia

duplicates removal. The 449 were screened using title and abstracts and, out of them, 118 were selected as pertinent and carefully reviewed. Articles without abstracts or unavailable from open source, reporting cases of contamination or pseudo-outbreaks, and articles that did not include human infections were excluded. Articles published in the observed period but regarding cases or outbreaks occurred before January 1, 2000 were also excluded. After this screening process 37 articles were identified and their full-text assessed for eligibility. Finally, 33 articles were selected and data were retrieved for further analysis. The other four articles were excluded because the same data they describe were reported in a different paper already considered in the review.

Therefore, during the period 2000-2014, 33 articles - reporting 34 episodes - have met inclusion criteria. Excluding year 2014, at least one episode has been described in each of investigated years.

According to the classification reported in the section of Methods, 24 (71%) episodes belonged to Class I and other 10 (29%) to Class II. Isolations reported include

genus *Legionella*, *Pseudomonas*, *Serratia*, *Ralstonia*, *Burkholderia*, *Klebsiella* and other pseudomonadaceae. The frequency of the episodes stratified by isolated pathogens is reported in Figure 1.

As shown in table 1 and 2, the results confirm that HCAIs can be associated also with liquids other than drinking water. The results regarding Legionnaires' disease (LD) will be described separately from those regarding other microorganisms, since, in Italy, this disease is the only one under special surveillance.

LRI and LRO due to Legionella spp.

In 14 episodes (42%) *Legionella pneumophila* (Lp) was the etiological agent ascertained or suspected. Table 1 shows the characteristics of each episode of LD and Class (I or II). Lp serogroup 1 (Lp1) is the most common, followed by Lp2-14, Lp3 and Lp5.

Most of episodes occurred in medical and surgical hospital wards (81%); some cases were described in dental clinics, thermal facilities and clinical homes for mentally disabled people. The type of water involved

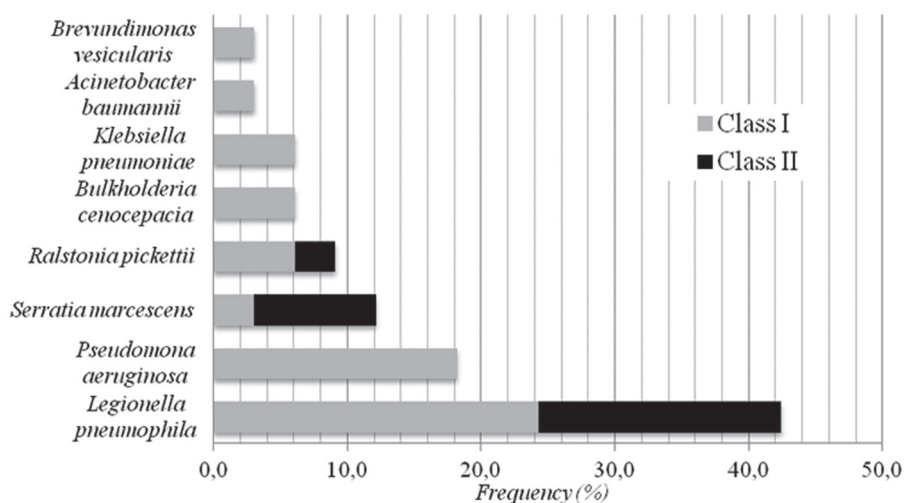


Figure 1 - Distribution (%) of HCAI episodes by isolated pathogens

included: drinking water, thermal water, water from cooling towers and from a tank for water birthing.

As shown in Table 1, at least one episode of LRI or LRO has been described each year, but 2014, during the period in exam.

In 2000 a case of LD due to Lp1 occurred in a 7-day old neonate after water birth in hospital. Since Lp1, responsible of child infection, was only isolated from the hospital pool water for water birthing, the authors conclude that the infant acquired the LD by prolonged delivery in contaminated water, perhaps by aspiration (18).

In the same year three surveillances were performed and results are reported in the literature. From 2000-2002, in a large hospital in Piedmont 9 cases of LD due to Lp1 occurred. The molecular investigation by ribotyping demonstrated the nosocomial origin (19). Other two cases of Lp1/*Philadelphia* were described in Campania, where the pulsed-field gel electrophoresis (PFGE) analysis showed high homology between the profiles of Lp1/*Philadelphia* isolated from both the patients and those from water samples (20). Finally, from 2000 until 2009 an environmental surveillance was carried out in Apulia by water sampling of 129 healthcare facilities after a risk assessment. *Legionella spp* was found in 102 (79.1%) of the healthcare facilities and in the 33.9% of the water samples. The most common environmental isolates were Lp1, Lp2-14 *L species*. In the same surveillance period 9 nosocomial cases were reported (21). The authors highlight the importance of the predictive value of a risk analysis and of the subsequent environmental microbiological tests, adding that laboratory diagnosis of LD cannot be excluded only on the basis of a single negative test (21). Only one case was nosocomial, confirmed by molecular investigation. This LD case, caused by Lp5, happened in 2001. Genotype comparison through PFGE between the strain isolated from the patient and those isolated from the

two different wards where the patient had been, both contaminated by Lp5, allowed the source to be identified (21).

During a surveillance (2001-2004) for nosocomial LD in Latium, among 43 cases of nosocomial pneumonia, 1 case (2.3%) was identified as nosocomial LD. The results of environmental investigations led the authors to conclude that the low incidence of nosocomial cases of LD could be associated with a low percentage (<20%) of positive water samples per semester and with a low contamination level (<10² CFU/L) (22). It was not possible to genetically compare clinical and environmental isolates, because, as in several other investigations, the bacteria could not be recovered from the patient.

Between 2001 and 2005, during an active surveillance procedure performed in three hospitals in Southern Italy, 12 cases of nosocomial LD caused by *Legionella spp* were diagnosed. Lp1 and Lp5 were found (23).

An environmental surveillance carried on in Turin during the period 2001-2002 showed that 35 out of the 36 hospitals investigated were positive for Lp; in the same period only one case of LD was diagnosed by cultural isolation (24).

Some strains of Lp of clinical and environmental origin, isolated from 3 healthcare facilities in Milan (2003-2006), were analyzed using three molecular typing methods: PFGE, amplified fragment length polymorphism (AFLP) and sequence based typing (SBT). PFGE and AFLP showed the correlation between a clinical and environmental isolates. (25, 26).

In 2003 a LD case in a 74-year-old woman was diagnosed, no cultural specimens were available. During the incubation period, the woman had attended a spa for hydrotherapy with sulphurous water by aerosol and nasal irrigation; high levels of Lp were isolated from the water in the nebulizers and nasal irrigators. The water system resulted Lp free after a combined protocol of shock treatment with

chlorine dioxide and peracetic acid, thermal shock and the restructuring of the water system. The authors conclude that to prevent *Legionella* colonization, disinfection treatment is effective if associated with carefully selected materials, good circuit design, and good maintenance practices (27).

In 2004, an outbreak of LD caused by Lp1/*Philadelphia* occurred in a hospital in Northern Italy and involved 8 cases with 2 deaths. Molecular typing by AFLP linked the outbreak to the contamination of the hospital water system and demonstrated the persistence of a predominant strain of Lp for 15 years (28).

The environmental strains of Lp1 obtained during routine surveillance carried on in 56 health care facilities in the Piedmont region (period 2004-2009) were typed using the monoclonal antibody MAb 3/1 of the Dresden Panel and compared with the number of cases that each healthcare facility reported during the study period. A statistically significant association between the presence of cases and colonization by MAb 3/1-positive Lp strains was demonstrated, suggesting that hospitals colonized by more virulent strains should be aware of the increased risk and should consider the opportunities of increasing their monitoring efforts and implementing more effective contamination control strategies (29).

Menacci et al. reported a case of a nosocomial LD by Lp3 occurred in 2010 in a 70 year old man with non-Hodgkin lymphoma. Diagnosis was carried out by culture and real-time polymerase chain reaction of bronchoalveolar lavage fluid. A hospital environmental investigation revealed that the hospital water system was highly colonized by Lp1, Lp3, Lp4 e Lp8. After the preventive measures adopted, no further cases of LD have been observed. Molecular typing of Lp clinical and environmental isolates was carried out by using the AFLP method, and the genomic profile of the Lp clinical strain matched with that of the Lp3 strain (30).

A confirmed fatal case of nosocomial LD due to Lp3 Oida strains, in a 63-year-old female patient, was reported in 2010 (31). In the study the source of infection was determined by using nested-SBT on post-mortem tissue samples, in absence of clinical isolates. Environmental strains were typed by SBT. AFLP was also used to type *Legionella* isolates.

Finally, in 2011, LD was diagnosed in an 82-year-old woman admitted to the intensive care unit. A Lp1 strain, isolated from the bronchial aspirate of a patient who received treatment in a dental clinic, was compared with environmental strains collected from the water of the same clinic using three different typing methods, which could demonstrate a homology between them. Monoclonal antibody typing (MBT) ascribed all the strains to subgroup Benidorm, one of the most virulent; SBT showed the same rare sequence type (ST 593) and AFLP showed identical genomic patterns (32).

LRI and LRO due to other microorganisms

As shown in Table 2, the review allowed to identify other 19 episodes, most of which (74%) fall in class I. Water caused the outbreak in 32% of episodes, while the remaining ones have been determined by several other fluids.

With regard to HCAI caused by *Pseudomonas aeruginosa*, this agent was involved in 6 (18%) outbreaks; the total cases accounted at 54 all in class I, 9 of which (17%) died.

In 2002, an outbreak of *P aeruginosa* infection in 6 patients who shared, during different periods, the same 2 rooms of a Bone Marrow Transplantation Unit (BMTU) of a Hospital in Florence, was reported. AFLP analysis of isolates from patients and from the showers' water suggested a common environmental source of infection (33).

Prospero et al. reported an outbreak of *P aeruginosa* catheter-related bloodstream infection (CRBSI) occurred in 2004 in a large

hospital in Ancona. The environmental strain was isolated from a mixture of heparin and saline solution. Clinical and environmental isolates were compared at PFGE, showing that the outbreak was due to a single clone of *P aeruginosa* (34).

In 2005, 9 patients developed *P aeruginosa* bloodstream infection (BSI) in a neurosurgical intensive care unit (NSICU) of Rome during a two-month-period. Environmental strains were isolated from a plasma-expander solution and tap water. All clinical and plasma-expander strains displayed the same PFGE pattern, whereas the water strains were unrelated to the outbreak (35).

During the period July 2005 - June 2007, in the NICU of a University hospital in Naples, 135 neonates became colonized by *P aeruginosa*; among them 11 developed a severe infection. PFGE analysis showed a homology among the profiles of clinical and environmental strains (36).

In 2006 six severe cases of *P aeruginosa* infections were reported in the Hematopoietic Stem Cell Transplant (HSCT) of a hospital in Florence. AFLP analysis of the isolates from the six patients showed the clonality of four strains; moreover, an isolate obtained from a soap dispenser located in the anteroom showed a significant molecular similarity (dice index higher than 0.93) with the four clinical strains (37).

In 2007 an outbreak of *P aeruginosa* severe infections was described in a hospital of Rome. It occurred in the HSCT Unit and involved 18 patients, 4 of whom died. The clonal relationship between *P aeruginosa* isolates was assessed by means of Random Amplified Polymorphic DNA (RAPD) analysis, macrorestriction analysis by PFGE, and Multilocus Sequence Typing (MLST). Of the eighteen cases, five constitute a significant molecular cluster of infection. A *P. aeruginosa* strain with the same genetic fingerprint and sequence type (ST175) as the strains of clinical origin was isolated

from a heavily contaminated triclosan soap dispenser. The authors conclude that the triclosan soap dispenser acted as a common continuous source of *P aeruginosa* infection. Since *P aeruginosa* is intrinsically unsusceptible to triclosan, the use of triclosan-based disinfectant formulations should be avoided in those healthcare settings hosting patients at high risk of *P aeruginosa* infection (38).

Serratia marcescens (*S marcescens*) has been reported in 4 outbreaks (12%), mainly occurred in neonatal ICUs (75%); total cases accounted at 63 (6 class I and 57 class II), 5 of whom died (8%).

In 2004, an outbreak of 6 cases of bloodstream infection due to *S marcescens* occurred in a surgical ward of a hospital in Brescia (39). Four patients had severe sepsis, but there were no deaths. The isolates were resistant to tetracycline and amoxicillin-clavulanic acid. Although a definite source for the outbreak was not identified, the Authors speculated that the lack of adherence to hand hygiene practices may had led to contamination of bags of total parenteral nutrition solution.

In 2007, Ligozzi et al report the comparison of three molecular methods used to investigate an outbreak of *S marcescens* infections in a neonatal intensive care unit (NICU) in Verona (40). Six patients developed infections; one experienced bacteremia, one conjunctivitis, one conjunctivitis and urinary infection, two urinary infection, and one umbilical wound infection. *S marcescens* was isolated from the milk samples and in three cases the isolates had a PFGE pattern comparable to clinical isolates. Moreover, the isolates from the NICU presented the same antimicrobial susceptibility profile and resulted resistant to ampicillin, amoxicillin-clavulanic acid, and cefuroxime.

In April 2011, an outbreak of *S marcescens* infections/colonisations occurred in the neonatal intensive care unit of Pescara

General Hospital. Rapid microbiological investigations led to the identification of five cases of likely cross-transmitted sepsis. Two low birth weight neonates died. The environmental investigation detected *S marcescens* from two soap dispensers. Hand carriage, although not demonstrated in this outbreak, was the most likely way of spread to and from soap dispensers (41).

Risk factors associated with *S marcescens* HCAI were evaluated by a 10-years (2003-2012) retrospective case-control study in a NICU of a tertiary level hospital in Modena. *S marcescens* was identified in 127 neonates: 43 developed infection and 3 died. Seven clusters were recorded due to 12 unrelated clones, which persisted for years in the ward, although no environmental source was found. Genotyping was effective in tracing the evolution and dynamics of the clones demonstrating their long-term persistence in the ward (15).

Three outbreaks due to *Ralstonia pickettii* (*Rp*) were also reported, accounting for 9% of episodes. The total cases described are 41 (13 class I and 28 class II); among them two patients died (5%).

The first outbreak occurred in 2001 in Perugia, involving 4 patients. The patients showed a systemic inflammatory response syndrome. PFGE and RAPD were used to investigate the relatedness of *Rp* isolates. Among the isolates with PFGE type A profile, RAPD identified strains R1 and R2. The source of infection was identified in a contaminated parenteral nutrition solution (42). The isolates were resistant to aminoglycosides and aztreonam.

In the same article, another outbreak due to *Rp*, occurred in 2002 in the same hospital, was described; it involved 9 patients, 2 of whom died; identical PFGE profiles and RAPD patterns have been observed. In this case the source of infections was identified in the heparin solution used to flush the central venous catheter (42).

Between 2001 and 2002, 28 cases of sepsis were reported in a Nephrology Unit of Como. *Rp* was isolated from the biofilm inside the equipment for hemodialysis, probably due to a contaminated liquid solution used for parenteral infusion (43).

Bulkholderia spp has been reported in 2 outbreaks (6%). The total cases of HCAI due to *Bulkholderia spp* are represented by 51 invasive infections, with 6 deaths.

A catheter-related outbreak of bacteraemia involving 38 patients in two haemodialysis units was reported in Verona in 2004-2005. *Burkholderia cenocepacia* (*B cenocepacia*) strains were isolated from human blood and from an individually wrapped disinfection napkin soaked in quaternary ammonium that was contained in a commercially available, sterile dressing kit used to handle central venous catheters. Microorganisms isolated from blood cultures and from the napkin were identified by standard procedures and confirmed as *B cenocepacia* by molecular analysis. Using PFGE analysis, the clinical isolates were closely related to the *B cenocepacia* isolated from the napkin (44).

A *B cepacia complex* outbreak occurred among ventilated non-cystic fibrosis patients in an ICU in Modena: 33 colonized and 13 infected patients were involved, 6 of them died. Molecular investigations conducted by RAPD demonstrated that the outbreak was associated with a contaminated mouthwash (45).

Two outbreaks (6%), attributed to *Klebsiella pneumoniae* (*K pneumoniae*), have involved 25 patients, causing sepsis and other severe pathologies. The first one regards the neonatal ICU of a teaching hospital of Naples where several outbreaks of *K pneumoniae* occurred from 1996 to 2004. Molecular typing of *K pneumoniae* isolates identified three distinct PFGE profiles. Strains of PFGE profile A were isolated during an epidemic in 1996, while isolates of PFGE profiles B and C were sequentially

isolated from September 2002 to December 2004, when 233 colonizations and 19 infections by *K pneumoniae* occurred. PFGE demonstrated the correspondence between environmental and clinical strains (46).

The second outbreak refers to six cases of primary bloodstream infections sustained by ampicillin/piperacillin-resistant *K pneumoniae*, observed over a two-month period in a NICU in Bologna. In order to determine genetic relatedness among strains, repetitive extragenic palindromic PCR (Rep-PCR) was applied. The results were confirmed by arbitrary primed polymerase chain reaction (AP-PCR) and RAPD. Rep-PCR confirmed that isolates of *K pneumoniae* from all six infants and from the saccharose solution belonged to the same genotype. All the *K pneumoniae* isolates obtained from the clinical samples had the same antibiogram, showing resistance to ampicillin and piperacillin (47).

Finally, 2 episodes due to other *pseudomonadaceae* have been described.

In 2005 an outbreak of *Acinetobacter baumannii* (*A baumannii*) occurred in the ICU of a hospital in Rome. The outbreak involved 14 patients whose isolates, most frequently recovered from bronchoalveolar lavage, were multidrug-resistant. *A baumannii* strains with a similar antibiotic susceptibility pattern were isolated from the environment. PFGE identified a single clone from both the clinical and environmental isolates (washbasin and other surfaces). During the ICU stay, 4 patients died, though none of the deaths was directly attributable to *A baumannii* infection. (48).

A case of meningitis due to *Brevundimonas vesicularis* (*B vesicularis*) has been described in 2006 in a 24-year-old male, without any previous diseases, hospitalized for concussion as a result of a fight, after he underwent a neurosurgical operation. *B vesicularis* was identified in the washbasins and water taps of the rooms in which the patient stayed (49).

Discussion

The present review allowed the selection of 34 episodes describing HCAs associated to liquid vehicles in Italy during the period 2000-2014, causing about 400 cases of infection. If we consider the limits of the research methods used and the awareness that large part of HCAs are not reported in literature, it is clear that the burden of this phenomenon is by far greater. The large majority of articles refers to hospital wards where patients with high risk of infections are usually admitted. Therefore, special attention in the procedures and their respect should be paid in these settings. Furthermore, correct physical design of hospital is an essential component to minimize the risk of infection transmission. For example, Hota et al (50), describing the placement and role of sinks as source of an ICU HCAs outbreak, showed that, although proximity of handwashing stations to the bedside is important, its placement too close can be a risk as well. The shallow depth of the basin resulting in splashing of contaminants from the drain to surfaces adjacent to the sink and directly onto the patient was the key factor resulting in this outbreak of serious infections and fatalities. Based on this and other evidences, the *US Guidelines for Design and Construction of Hospitals and Healthcare Facilities, 2010* (51) recommend to design handwashing stations in the ICUs and other wards in a way that could reduce the risk of microorganisms' spread (51, 52).

While many articles describe single or epidemic cases occasionally diagnosed, it is interesting to underline that some other articles report cases of HCAs, that have been identified in the context of local routine surveillance systems (19, 20, 25, 26). Therefore, building up surveillance systems can be considered as an effective control procedure, and their implementation should be expanded and improved. This is in line

with other findings according to which, an effective clinical strategy of HCAI prevention includes aggressive clinical surveillance with *ad hoc* further investigation in case of detection of every single case (2).

With regard to the sources of infection, water should be considered of particular relevance due to the numerous occasions of exposure (5), but some other liquids can be considered efficient vehicles of pathogens. This evidence should be taken into account when identifying the procedure of prevention, especially if we consider that even disinfectants are listed among these liquids. Moreover, in some cases the isolated pathogens were investigated for their antimicrobial resistance, allowing to identify the most appropriate therapy to be adopted according to the HCAI agents circulating (46).

Another interesting aspect to consider is the healthcare facilities where the HCAI were acquired; as a matter of facts, besides the hospitals, other contexts, such as the spa facilities, seem to have a role in the presence of some risk factors, for example water temperature can encourage the growth of microorganisms causing HCAI. Moreover, guests attending this type of facilities can be at risk for infection when are undergoing inhalation therapy. A very accurate management of these facilities is, therefore, required to avoid infections such as those caused by *Legionella* spp. and not always national guidelines provide clear suggestions on their management; on the contrary, prevention systems should be put in place for the well-being of clients and patients (53).

With regard to etiological agents, a large number of articles are dedicated to *Legionella* spp, but the number of cases is not always as high as in HCAI caused by other pathogens. Although, many other microorganism were identified in our review, we did not find any report regarding outbreak caused by *Mycobacterium avium*, currently considered

the causal agent of the majority of non-tuberculous mycobacteria infections and the most prevalent *Mycobacterium* in drinking water (54). Moreover, information available on disease incidence for opportunistic plumbing pathogens indicates other emerging microorganisms, like *Naegleria fowleri*, a brain-eating amoeba that prefers warm aquatic environments, recently detected in plumbing and linked to deaths in the southern United States (55).

With regard to diagnosis, in particular LD shows some difficulties (often it is not a routine laboratory practice, urine antigen emission is not constant, the antibody response is slow, etc.), therefore a special attention must be paid. Moreover, the selected articles demonstrated that the great majority of LD cases are cause by Lp1; this result is consistent with those provided by NSS (56), but the problem is that the majority of cases reported are diagnosed by urinary antigen detection only; therefore, serogroups different from Lp1 are not always detected. On the contrary, the isolation and identification of the etiological agent is rarely performed, but it is fundamental to reach the source of infection and to program the necessary disinfection measures, so limiting the spread of the disease to both patients and health staff (21).

The cultural methods should always be considered the first choice to allow the comparison between clinical and environmental strains. With regard to this issue, different methods have been described. In order to correlate clinical and environmental strains, phenotyping methods such as serotyping, biochemical tests, antibiotic susceptibility, and phage typing were mainly used in older studies. Unfortunately, these methods appear to be weakly discriminatory in distinguishing closely related strains (57). Molecular typing techniques were performed more recently, when the usefulness of such methods for the 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 (57).

As previously reported by Falkenham et al, there are several traits shared by the microorganisms that we found as water related HCAI agents: the ability to adhere to surfaces and form biofilms that make the microorganism also more resistant to disinfectants, survival at high temperatures, and growth in free-living phagocytic amoebae (54). These factors impact directly the human exposure during health care procedures. Furthermore, the ageing of hospital buildings and the need of continuing structural and technology adjustment justify the presence, almost continuous, of yards in healthcare facilities, that interact in many ways with the hospital, exposing patients to further risk factors (58). Therefore, only by a multiple approaches of engineering and hygiene measures as well as surveillance and management for hospital water it is possible to reduce the risk of contracting water related HCAI (58). Hospitals should have prospective water safety plans that include preventive measures, as prevention is preferable to remediation of contaminated hospital water distribution systems (2). Moreover, it is essential for

healthcare personnel to know reservoirs and transmission pathways of these infections for adopting the correct control measures of prevention (59), especially because, as showed by our review, not only water, but other liquids are responsible for HCAI onset; therefore, the respect of good practice should be extended to all procedures that can pose at risk the patients.

Riassunto

Le sostanze liquide possono costituire un efficiente veicolo d'infezioni correlate all'assistenza? Una revisione dei casi riportati in Italia nel period 2000-2014

Introduzione. Tra le infezioni correlate all'assistenza (ICA), le più studiate sono quelle veicolate dall'acqua, sia perché maggiormente suscettibili di piani di controllo, sia per la grande quantità di acqua utilizzata in ambiente ospedaliero. Tuttavia, anche altri liquidi possono essere associati ad ICA; perciò, il presente studio riporta i risultati di una revisione scientifica sulle ICA associate a veicoli liquidi verificatesi in Italia nel periodo 2000-2014.

Metodi. La revisione della letteratura si è incentrata sui casi di ICA, sia in forma epidemica che sporadica, in accordo con le definizioni ufficiali fornite nel 2011 da Organizzazione Mondiale della Sanità e Centri Americani per il Controllo delle Malattie. Sono stati considerati tutti gli articoli pubblicati in riviste scientifiche con dimostrata associazione (epidemiologica e/o molecolare) tra ICA e veicoli fluidi. Sono stati esclusi casi di ICA associati a trasmissione parenterale (tramite sangue e derivati).

Risultati. Nel periodo 2000-2014, sono stati descritti 34 episodi di ICA associati a veicoli liquidi, per un totale di circa 400 casi di malattia. I microorganismi interessati appartengono ai generi *Legionella*, *Pseudomonas*, *Serratia*, *Ralstonia*, *Burkholderia*, *Klebsiella* e altre pseudomonadaceae. I risultati hanno confermato che le ICA possono essere associate anche ad altri liquidi oltre l'acqua e che la gran parte dei casi riguardava reparti con pazienti ad alto rischio infettivo.

Discussione. La revisione ha evidenziato un alto numero di casi di ICA; tuttavia, considerando che non tutte le ICA costituiscono oggetto di articoli scientifici, si può immaginare quale sia la reale dimensione del problema. Molti casi sono stati identificati attraverso sistemi locali di sorveglianza e questo dimostra il ruolo centrale della sorveglianza nel controllo delle ICA. Così come appare centrale il ruolo dell'isolamento e identifi-

cazione del microrganismo responsabile di ICA, al fine di identificarne l'origine e programmare i più corretti interventi di controllo. Pertanto, in considerazione dei vari aspetti emersi dalla revisione, solo attraverso un approccio multidisciplinare è possibile il controllo di questo fenomeno.

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