

# Microbiological investigation of a nosocomial case of *Legionella pneumophila* pneumonia associated with water birth and review of neonatal cases

**Indagini microbiologiche su un caso di polmonite nosocomiale da *Legionella pneumophila* associata a parto in acqua e revisione dei casi neonatali**

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## INTRODUCTION

Legionnaires' disease occurs in sporadic, endemic and epidemic forms. *Legionella pneumophila* infection accounts for 5-10% of pneumonia cases in adults, but less than 1% in children [1]. Nosocomial legionellosis, that often affect patients at high risk, have been described in adults, but rarely in children and neonates [1-5]. The disease in normal children is characterized by fever, cough and progressive respiratory distress. More than half of the cases reported in neonates are immunocompromised or have systemic disease that may have predisposed them to *Legionella* infection [5]. Legionellosis is usually acquired by inhalation of contaminated aerosols and sometimes by aspiration of contaminated water in association to nasogastric tube use [6].

We previously report a case of *L. pneumophila* serogroup 1 pneumonia in a 7-day old immunocompetent neonate after water birth [7]. The neonate, discharged from general hospital 4 days after birth, was readmitted 3 days later after the appearance of fever and dyspnea. Because of the persistence of infiltrates by chest radiograph, despite progressive clinical improvement after therapy, he was transferred to the pediatric department at the age of one month. The laboratory tests, performed at that

time, demonstrated *Legionella* infection. As sample for culture was not available and, consequently, strains to type for epidemiological purposes, further laboratory tests were necessary in order to recognize the source of infection. In this study we describe the microbiological investigations performed in neonate and environment to clearly demonstrate the nosocomial origin of the infection and to trace the mode of transmission. A review of neonatal cases of legionellosis is also presented.

## MATERIALS AND METHODS

*Laboratory diagnosis of Legionella infection.* Antibody titers were tested in 3 sequential neonate sera collected at 26, 33 and 51 days after the onset of symptoms (late december 1999). The methods used were: 1) indirect immunofluorescence (IFA) [8] with phenol killed antigen of *L. pneumophila* serogroup 1 Philadelphia 1 and polyvalent antigens (Poli I: *L. pneumophila* serogroup 1 to 3; Poli II: *L. pneumophila* serogroup 4 to 6; Poli III: *L. pneumophila* serogroup 7 to 10; Poli IV: *L. bozemanii* serogroup 1 and 2, *L. micdadei*, *L. dumoffii*; Poli V: *L. gormanii*, *L. jordanis* and *L. longbeachae*), kindly provided by M. Castellani Pastoris (Legionella National Center, Istituto Superiore di

Sanità, Rome, Italy) [9]; 2) microagglutination (MA) using home-made antigens of *L. pneumophila* serogroup 1 Philadelphia 1 and *L. pneumophila* serogroup 6 (Chicago 2). In order to evaluate antibody titers against monovalent antigens, the sera were subsequently tested by IFA with the following home-made formalized antigens: *L. pneumophila* serogroup 2 (Togus 1), *L. pneumophila* serogroup 3 (Bloomington 2), *L. pneumophila* serogroup 4 (Portland 1), *L. pneumophila* serogroup 5 (Cambridge 2), *L. pneumophila* serogroup 6 (Chicago 2), *L. pneumophila* serogroup 1 monoclonal subtype Knoxville-1 and France 5811/Allentown-1 (the last two strains were isolated from the water supply of the hospital where the neonate was born). Legionella urinary antigen was performed by EIA Biotest (Dreieich, Germany) on 7 unconcentrated and concentrated [10] urine samples, collected from the first to the fourth month (32, 33, 38, 47, 54, 74, 111, 181 days after the onset of symptoms). Respiratory samples were not available for Legionella culture. Antibody titers against *L. pneumophila* serogroup 1 were also tested in a serum sample of the neonate's mother, who always was healthy, in order to determine possible antibodies acquired transplacentally from mother to child.

*Environmental investigations.* As the incubation period of legionellosis (2-10 days) strongly suggests that the pneumonia occurring in the 7-day old neonate was nosocomial, Legionella culture was performed from the tap water of the neonate home as well as from the water supply of the hospital where the neonate was born. The municipal water of the house (a two-floor cottage) is heated by a 100 L independent hot water heater. Five liters of hot and cold tap water were collected from 3 basins in February 2000. Samples were also obtained by swabbing the basins faucet and resuspending the swab in 100 mL of hot water. 300 mL of tap water with a little quantity of aromatic oils from 5 humidifiers were also collected and the containers swabbed. Ten liters samples of hot and cold tap water were collected again from basins in May 2000. Temperature and free chlorine were determined immediately after water collection. In the hospital the municipal water is heated by 5 vertical hot water heaters (5000 L), collected in a pipe and distributed through the building; a recirculating system of the hot water is present. Five liters water samples were collected in May 2000 from the central water heaters and from

different points of 3 gynaecological surgery room, including waterbirthing one, and from the patient's room.

Samples, processed as previously described, were concentrated by filtration (0.2 µm cellulose acetate membrane filters) and resuspended into 5 mL of the same water [11]. Aliquots (0.1 mL) of untreated, heat-treated and acid-treated were plated on BCYE α, BMPA α and MWY media. GVPC was also used for humidifiers water [12]. Colonies with typical morphology, gram-negative staining and requiring L-cysteine for the growth, were identified by latex agglutination (Oxoid, UK), by IFA (Scimed, New Jersey, U.S.A.) or by agglutination with polyclonal rabbit antisera (Biogenetics, Italy). *L. pneumophila* serogroup 1 strains were subtyped using monoclonal antibodies (Dresden panel, provided by J.H. Helbig) [13]. Legionella spp. isolates were typed by Dr R. Benson (CDC, Atlanta). The total bacteria count of all water samples was performed on standard plate count agar at 37° C for 3 days and at 25° C for 5 days.

*PCR.* Direct detection of Legionella spp. and *L. pneumophila* (*mip* gene) from the patient's home water was also performed by PCR using EnvironAmp Legionella PCR (Perkin Elmer, U.S.A.), following manufacturer instructions, but samples were previously treated before amplification as follows:

- 1) 10 mL of water samples of May 2000 were filtered (0.45 µm cellulose acetate membrane);
- 2) 300 µL of concentrated filtered water of the same samples, used for culture, were treated with 300 µL of lysis reagent;
- 3) 300 µL of the concentrated filtered water from humidifiers and from tap swabbing samples of February 2000, were treated with 300 µL of lysis buffer.

## ■ RESULTS

The results of laboratory test on the neonate sera are shown in Table 1. Significant antibody titers were detected against *L. pneumophila* serogroup 1, by IFA (1/256) and MA (1/4096) and against Polivalent I and II antigens by IFA on the sample collected at 26 days after symptoms' onset; a marked decrease was observed 25 days later. Antibody titers against *L. pneumophila* serogroup 6 showed a decrease from 1/128 to <1/32, while against *L. pneumophila* serogroup 2 to 5 were negative. Titers against *L.*

*pneumophila* serogroup 1 strains of the two monoclonal subtypes showed the same results as reference strain. Legionella urine antigen was positive on 7 repeated unconcentrated and concentrated urine samples, collected from the first to fourth month, and became negative at the next control at sixth month. Antibody titers against Legionella were negative in the serum of the neonate's mother.

The results of Legionella culture from samples collected on the patient's home are shown in Table 2. The strains isolated from cold tap water were identified as *L. spiritensis*. Only one positive sample (10 cfu/L) was found on Feb-

ruary 2000, 3 positive samples (10-225 cfu/L) on May 2000, when 10 liters of water were collected. All the samples collected by tap swabbing were negative. Humidifiers water was negative, but many colonies of eterotrophic bacteria were present on the selective media plate; growth inhibition by essential oils was also observed. All PCR were positive by reverse dot blot for Legionella spp., but not for *L. pneumophila* serogroup 1 DNA.

The results of culture from hospital water supply are shown in Table 3. All the hot water samples were positives and yielded *L. pneumophila* serogroup 1 strains at 300 to 2000 cfu/L. The

**Table 1 - Antibody titers against Legionella in neonate sera**

Serum no.	Days after symptom's onset	IFA <sup>a</sup> titers					MA <sup>b</sup> titers	
		Lp <sup>c</sup> 1	Lp 2, 3, 4, 5	Lp 6	P <sup>d</sup> I, II	P III, IV, V	Lp 1	Lp 6
1	26	256	< 32	128	256	<32	4096	128
2	33	256	<32	128	256	<32	4096	64
3	51	32	<32	16	32	<32	512	8

<sup>a</sup>IFA = Indirect fluorescence antibody assay; <sup>b</sup>MA = Microagglutination assay; <sup>c</sup>Lp = *L. pneumophila*; <sup>d</sup>P = Polivalent antigens

**Table 2 - Legionella isolation from water samples on the patient's home**

Sample site	Sample No. 1	Sample No. 2	Sample No. 1	Sample No. 2
Bathroom cold tap water	9	18.5	10	225
Bathroom hot tap water	67	61	-	-
Kitchen cold tap water	7.5	18	-	10
Kitchen hot tap water	69.5	65	-	-
Laundry cold tap water	7	19	-	10
Laundry hot tap water	63	62	-	-

**Table 3 - Results of Legionella isolation from hospital water samples**

Source	Sample site	Water T <sup>a</sup> (°C)	Legionella (cfu/L)	Tbc <sup>b</sup> 37°C	(cfu/mL) 25°C
Hot water	Income pipe collecting water - CHSc	51	1000	2	<1
Hot water	Tank No. 5 outlet - CHSc	51	2000	<1	<1
Hot water	Tank No. 2 outlet - CHSc	46	2000	<1	<1
Cold water	Faucet after demineralization system - CHSc	10	<10	<1	4
Hot shower-head water	Waterbirthing tank - gynaecological surgery room A	48.5	400	8	10
Hot tap water	Washbasin - gynaecological surgery room A	48	350	8	4
Hot tap water	Washbasin - gynaecological surgery room B	47	300	30	25
Hot tap water	Washbasin - gynaecological surgery room C	47	800	80	10
Hot tap water	Washbasin of patient's room - obstetrics ward	48	900	50	70

<sup>a</sup>T = Temperature; <sup>b</sup>Tbc = Total bacteria count; <sup>c</sup>CHS = Central heating system

temperature of hot water ranged from 46° C to 51° C (mean value: 48.3° C). The environmental strains of *L. pneumophila* typed by Dresden panel were of two monoclonal subtype of Pontiac subgroup (Knoxville-1 and France 5811/Allentown-1).

## ■ DISCUSSION

Legionella infection occurs mostly in adult patients. Review of pediatric literature suggests that *L. pneumophila* is a rare cause of pneumonia in children [2-4]. Almost all the infections (92.8%) in these subjects are of nosocomial origin [3]. Most patients (83.8%) have predisposing risk factors for acquiring the infection and immunosuppression is the first one.

The incidence among neonates is unknown. The number of cases is presumably underestimated, most likely because Legionella is not considered as a causative agent of neonatal pneumonia. Pediatricians are not aware of the possibility of legionellosis and do not request special laboratory tests for Legionella. Other reasons for underrecognition of the infection in children and in neonates are the difficulty of the laboratory diagnosis and the clinical variability of legionellosis in these patients. Eleven sporadic cases of neonatal pneumonia have been described in the literature and 7 had a fatal outcome [5, 14, 15]. Ten were nosocomial and in 5 of these cases Legionella was documented in the hospital environment [14, 16-19]. Molecular methods confirmed the identity of the clinical and environmental isolates in one case, associated to incubator humidifier [18], while in another case the confirmation was obtained by monoclonal antibodies subtyping [16]. *L. pneumophila* serogroup 1 was the predominant serogroup reported [18-22]. Previous nosocomial cases in infants were associated to contaminated water at various stages of their hospital stay [14, 16, 17, 19], only one neonate was infected by inhalation of aerosol released from the humidifier within the incubator [18]. In the present study we demonstrated the first nosocomial case of *L. pneumophila* pneumonia in neonate following water birth. The water supply of the hospital where the neonate was born, and particularly the pool water for waterbirthing, were contaminated by *L. pneumophila* serogroup 1 (300-2000 cfu/L). On the contrary, *L. spiritensis* strains were only isolated from cold water (10-225 cfu/L) of the patient's house

in two instances. The temperature of the first cold water samples was unusually low (7-9° C) for growth. Although Legionella has been isolated from water at temperatures ranging from 5.7° C to 63° C [23], the bacterium probably only multiplies actively from 20° C to 45° C. The positive results of PCR for Legionella spp. from home humidifiers, without strains isolation, suggested that the growth was inhibited by heterotrophic bacteria or essential oils.

Significant antibody titers against *L. pneumophila* serogroup 1 were found by IFA and MA in the neonate's sera. Antibody titers against *L. pneumophila* serogroup 6 were lower. This may be due to cross-reacting antibodies, but a dual infection can not be completely excluded. Even if environmental water samples collected in the general hospital did not evidence *L. pneumophila* serogroup 6, colonies of this serogroup, together with serogroup 1, were isolated 5 months before the neonate birth. Water disinfection (super heat-and-flush) was then performed and microbiological analysis of hospital water samples, collected 3 months before the newborn nosocomial case, did not evidence Legionella. The possibility that antibodies could be acquired transplacentally in the newborn was excluded, because the serodiagnosis was negative in the mother's neonate who always was healthy [24]. The fact that the hospital water supply was contaminated by the same serogroup of *L. pneumophila* responsible for child infection strongly suggests that he was infected following prolonged delivery in contaminated water, perhaps by aspiration. After the notification of this nosocomial case, the birthing pool of the general hospital was immediately disinfected, a filter was introduced in the shower-head of the water tub and more attention was paid to infection risk during waterbirthing also in the other hospitals.

The legionellosis is usually acquired by inhalation of contaminated aerosol. The aspiration is described in patients with nasogastric tube use [6]. Seven of 9 previous nosocomial cases of legionellosis in neonates were predisposed to Legionella infection because of underlying disease and prematurity [16-20, 22, 25, 26]. The case reported here concerns a full term immunocompetent newborn with normal body weight. No incubator was used for this neonate in the hospital. Aspiration during prolonged delivery may be the mode of transmission, but the exposure dose remains unknown.

Contaminated whirlpool baths have been re-

ported as a source of legionellosis in adults [27]. Birthing pool have been associated to a community-acquired fatal case of *L. pneumophila* pneumonia in a neonate after water-birth in a home bathtub in Japan [28]. The diagnosis was made on post-mortem lung by PCR and IFA (*L. pneumophila* serogroup 6). From water samples, collected from the home bathtub, high number of Legionella colonies (species not identified) was found.

Early diagnosis and institution of appropriate therapy for legionellosis are critical determinant of outcome. Delay in initiation of specific therapy results in poorer prognosis [29]. Mortality reported in newborn (60%) was higher than in adults and survival directly depended on administration of erythromycin [5]. In this study the newborn was early treated with clarithromycin and he survived.

The reported incidence of nosocomial pneumonia is directly correlated with two factors: the ready availability of specialized diagnostic test and the presence of Legionella in the hospital water supply [30]. Because of the rarity of this infection in neonates and because essentially all cases reported were nosocomial, environmental surveillance should be promptly activated and culture for Legionella should be performed. There are however conflicting opinions regarding role and efficacy of routine surveillance of water for Legionella contamination.

Water birth is increasingly being offered as an option, although concerns about associated in-

fection risk has been expressed [31]. Neonatal Pseudomonas sepsis has been also documented [32]. Post-natal surveillance of mothers and babies is required and infection control policies (pool maintenance, decontamination for Legionella and universal precautions) are highly recommended to prevent legionellosis transmission [31].

In conclusion, we reported the first nosocomial case of Legionella pneumonia in neonate following water birth. The newborn was infected during delivery in contaminated water, perhaps by aspiration. The demonstration was obtained by extended studies on child and environment, because clinical sample was not available for culture. Legionella infection should be suspected in neonates with nosocomial pneumonia and specialized laboratory tests should be early requested. As neonatal legionellosis may have a high fatality rate if unrecognized, pediatricians should be aware of this possible transmission route. Infection control policies should be implemented for water birth.

*Key words:* water birth, Legionellosis, nosocomial, newborn

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## SUMMARY

A case of *Legionella pneumophila* 1 pneumonia, confirmed by positive serology and urinary antigen, occurred in a 7-day old neonate after water birth in hospital. As respiratory samples were not available for culture, further microbiological investigations were performed on the neonate and the environment, in order to recognize the source of infection. The hospital water supply was contaminated by *L. pneumophila* 1 strains (300-2000 cfu/L) of two monoclonal subtypes of the Pontiac subgroup. *L. spiritensis* (10-225 cfu/L) was isolated from cold tap water of the patient's home. PCR from tap and humidifier water at

the patient's home was positive for *Legionella* spp, but not for *L. pneumophila*. As *L. pneumophila* 1, responsible for child infection, was only isolated from the hospital pool water for waterbirthing, we conclude that the infant acquired the nosocomial legionellosis by prolonged delivery in contaminated water, perhaps by aspiration. Infection control measures for waterbirthing are highly recommended. A review of neonatal cases of legionellosis is also presented. As this rare infection may have a high fatality rate if unrecognized, pediatricians should be aware of the possibility of legionellosis in the newborn.

Un caso di polmonite da *Legionella pneumophila* 1, confermato da sierodiagnosi e antigene urinario, è stato osservato in un neonato di 7 giorni, nato in ospedale con parto in acqua. Poiché non erano disponibili campioni per la coltura, sono state eseguite ulteriori indagini microbiologiche nel neonato e nell'ambiente per riconoscere la sorgente d'infezione. L'impianto idrico ospedaliero risultava contaminato da ceppi (300-2000 cfu/L) di *L. pneumophila* 1 appartenenti a 2 sottotipi monoclonali del sottogruppo Pontiac. *L. spiritensis* (10-225 cfu/L) è stata isolata dall'acqua fredda della casa del paziente. Il test PCR è risultato positivo per *Legionella*

*spp*, ma non per *L. pneumophila*. Poiché *L. pneumophila* 1 è stata isolata solo dall'acqua della vasca per parto dell'ospedale, si conclude che la legionellosi nosocomiale è stata acquisita dal neonato dopo parto prolungato in acqua contaminata, probabilmente per aspirazione di liquidi. Si raccomanda l'adozione di severe misure di controllo dell'infezione per la pratica del parto in acqua. In questo lavoro viene anche presentata una revisione dei casi neonatali di legionellosi. Poiché questa rara infezione presenta elevato tasso di letalità se non riconosciuta, i pediatri dovrebbero prendere in considerazione la possibilità di legionellosi nei neonati.

## REFERENCES

- [1] Edelstein PH. Legionnaires' disease, Pontiac fever, and related illness, In *Pediatric Infectious Diseases* (Cherry JD., Feigin RD., Eds) 3rd edn., 1992, pp 1141-1148. WB Saunders, Philadelphia.
- [2] Brady MT. Nosocomial legionnaires disease in a children's hospital. *J. Pediatr.* 115, 45-50, 1989.
- [3] Campins M., Ferrer A., Callis L. et al. Nosocomial Legionnaires' disease in a children's hospital. *Pediatr. Infect. Dis. J.* 1, 228-234, 2000.
- [4] Carlson NC., Kuskie MR., Dobyms EL., Wheeler MC., Roe MH., Abzug MJ. Legionellosis in children: an expanding spectrum. *Pediatr. Infect. Dis.* 9, 133-137, 1990.
- [5] Levy I., Rubin LG. Legionella pneumonia in neonates: a literature review. *J. Perinatol.* 18, 287-290, 1998.
- [6] Edelstein PH. Legionnaires' disease. *Clin. Infect. Dis.* 16, 741-747, 1993.
- [7] Franzin L., Scolfaro C., Cabodi D., Valera M., Tovo PA. *Legionella pneumophila* pneumonia in a newborn following waterbirth: a new mode of transmission. *Clin. Infect. Dis.* 33, e103-104, 2001.
- [8] Castellani Pastoris M., Ciarrocchi S., Di Capua A., Temperanza AM. Comparison of phenol- and heat-killed antigens in the indirect immunofluorescence test for serodiagnosis of *Legionella pneumophila* serogroup 1 infections. *J. Clin. Microbiol.* 2, 780-783, 1984.
- [9] Wilkinson HW. Hospital-laboratory diagnosis of Legionella infections. 1988. Centers for Disease Control and Prevention, Atlanta.
- [10] Domínguez JA., Manterola JM., Blavia R. et al. Detection of *Legionella pneumophila* serogroup 1 antigen in nonconcentrated urine and urine concentrated by selective ultrafiltration. *J. Clin. Microbiol.* 3, 2334-2336, 1996.
- [11] Franzin L., Castellani Pastoris M., Gioannini P., Villani G. Endemicity of *Legionella pneumophila* serogroup 3 in a hospital water supply. *J. Hosp. Infect.* 13, 281-288, 1989.
- [12] Dennis PJ., Bartlett CLR., Wright AE. (1984) Comparison of isolation methods for *Legionella spp*, In *Legionella. Proceedings of the 2nd International Symposium.* (Thornsberry C., Balows A., Feeley JC., Jakubowski W., Eds.) 1984, pp 294-296. American Society for Microbiology, Washington D.C.
- [13] Lück PC., Dinger E., Helbig JH. et al. Analysis of *Legionella pneumophila* strains associated with nosocomial pneumonia in a neonatal intensive care unit. *Eur. J. Clin. Microbiol. Infect. Dis.* 13, 565-571, 1994.
- [14] Ferrer Marcelles A., Garcia Hernandez F., Elcuaz Romano R., Tokashiki Tokumura N., Fernandez Y. Pneumonia caused by *Legionella* in a newborn infant. *An. Esp. Pediatr.* 30, 213-214, 1989.
- [15] Skogberg K., Nuorti JP., Saxen H. et al. A newborn with domestically acquired legionnaires disease confirmed by molecular typing. *Clin. Infect. Dis.* 35, e82-85, 2002.
- [16] Aubert G., Bornstein N., Rayet I., Pozzetto B., Lenormand P.H. Nosocomial infection with *Legionella pneumophila* serogroup 1 and 8 in a neonate. *Scand. J. Infect. Dis.* 22, 367-370, 1990.
- [17] Holmberg RE., Pavia AT., Montgomery D., Clark JM., Eggert LD. Nosocomial *Legionella* pneumonia in the neonate. *Pediatrics* 9, 450-453, 1993.
- [18] Lück PC., Helbig JH., Ehret W., Marre R., Witzleb W. Subtyping of *Legionella pneumophila* serogroup 1 strains isolated in Germany using monoclonal antibodies. *Zentralblatt. Bakteriol.* 277, 179-187, 1992.
- [19] Negre V., Chevallier B., Dournon E., Bidat E., Coignard S., Lagardere B. Nosocomial legionnaires' disease in children, preventive measures. *Arch. Fr. Pediatr.* 47, 43-45, 1990.
- [20] Green KA., Rhine WD., Starnes V., Ariagno RL. Fatal postoperative *Legionella* pneumonia in a newborn. *J. Perinatol.* 10, 183-184, 1990.
- [21] Horie H., Kawakami H., Minoshima K. et al. Neonatal Legionnaires' disease. Histopathological findings in an autopsied neonate. *Acta Pathol. Jpn.* 4, 427-431, 1992.
- [22] Womack SJ., Liang KC., Ilagan NB., Weyhing BT., Planas A. *Legionella pneumophila* in a preterm infant. A case report. *J. Perinatol.* 12, 303-305, 1992.
- [23] Fliermans CB., Cherry WB, Orrison LH, Smith SJ., Tison DL., Pope DH. Ecological distribution of *Legionella pneumophila*. *Appl. Environ. Microbiol.* 41, 9-16, 1981.
- [24] Silberg SL., Lawrence CH., Guthrie PJ. Transpla-

central transfer of *Legionella pneumophila* antibodies. *Pediatr. Infect. Dis. J.* 6, 925-927, 1987.

[25] Ahrens F., Hell W., Hollandt H., Lewald H., Paust H. Legionellosis in a newborn. *Monatsschr. Kinderheilkd.* 141, 711-713, 1993.

[26] Ferrer A., Elcuar RL., Gimenez-Perez M., Iglesias J., Fernandez-Perez F. Childhood legionellosis. *Enferm Infecc Microbiol Clin* 8, 278-281, 1990.

[27] Centers for Disease Control and Prevention. Legionnaires' disease associated with a whirlpool spa display - Virginia, September-October 1996. *MMWR* 46, 83-86, 1997.

[28] Nagai T., Sobajima H., Iwasa M. et al. Neonatal sudden death due to *Legionella pneumonia* associated with

water birth in a domestic spa bath. *J. Clin. Microbiol.* 41, 2227-2229, 2003.

[29] Health CH., Grove DI., Looke DFM. Delay in appropriate therapy of *Legionella pneumonia* associated with increased mortality. *Eur. J. Clin. Microbiol. Infect. Dis.* 15, 286-290, 1996.

[30] Stout JE., Yu VL. Legionellosis. *New Engl. J. Med.* 33, 682-687, 1997.

[31] Kingsley A., Hutter S., Green N., Speirs G. Waterbirths: regional audit of infection control practices. *J. Hosp. Infect.* 41, 155-157, 1999.

[32] Vochem M., Vogt M., Döring G. Sepsis in a newborn due to *Pseudomonas aeruginosa* from a contaminated tub bath. *N. Engl. J. Med.* 345, 378-379, 2001.