



## Water birthing: results from a 18 months-long microbiological surveillance

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### ABSTRACT

**Objectives:** The aim of our study was to monitor the effectiveness of the cleaning and disinfecting procedures used in the pool for pre-delivery courses and single tubs for water birthing at "Careggi" Hospital (Florence, Italy).

**Methods** We conducted a cross-sectional study collecting water samples and swabs. After microbiological analysis data were organized in a database and then exported for statistical analysis.

**Results:** We collected 15 water samples from the pool: *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococci* resulted negative. We collected 142 samples from the 4 tubs for the water birth. There was a statistically significant difference ( $p=0.01$ ;  $OR=0.28$ ) in the presence of *Staphylococcus* spp. in the two examined years: in 2017 their presence was significantly lower ( $N=5$ ) than 2016 ( $N=23$ ).

There was also a statistically significant difference ( $p=0.03$ ) in the presence of yeasts in the 2 examined years: in 2017 six samples resulted positive, in 2016 no one. Chi square test evidenced that the water discharge system was at a higher risk of being contaminated by staphylococci ( $p=0.01$ ,  $OR=3.08$ ).

**Conclusion:** A continuous monitoring and the implementation of training programs are important to increase the knowledge of the right cleaning procedures by operators and avoid infections.

**Keywords:** healthcare-associated infections, water, birth, delivery, surveillance

### SOMMARIO

**Obiettivi:** Lo scopo del nostro studio è stato monitorare l'efficacia delle procedure di pulizia e disinfezione utilizzate per la piscina dedicata ai corsi pre-parto e per le vasche usate per il parto in acqua all'interno dell'Azienda Ospedaliero Universitaria "Careggi" (Firenze, Italia).

**Metodi:** Abbiamo effettuato uno studio cross-sectional raccogliendo campioni d'acqua e effettuando tamponi. I dati derivanti dalle analisi microbiologiche sono stati poi inseriti in un database ed esportati per l'analisi statistica.

**Risultati:** Abbiamo raccolto 15 campioni d'acqua dalla piscina: *Escherichia coli*, *Pseudomonas aeruginosa* ed *Enterococchi* erano assenti. Abbiamo raccolto 142 campioni dalle 4 vasche per il parto in acqua. È stata osservata una differenza statisticamente significativa ( $p = 0,01$ ;  $OR = 0,28$ ) per la presenza di stafilococco spp. nei due anni esaminati: nel 2017 la sua presenza era significativamente più bassa ( $N = 5$ ) rispetto al 2016 ( $N = 23$ ).

È stata anche osservata una differenza statisticamente significativa ( $p = 0,03$ ) per la presenza di funghi nei 2 anni esaminati: nel 2017 sei campioni sono risultati positivi, nel 2016 nessuno. Il Test del chi quadrato ha evidenziato che il sistema di scarico dell'acqua era a rischio più elevato di essere contaminato da stafilococchi ( $p = 0,01$ ,  $OR = 3,08$ ).

**Conclusioni:** Un monitoraggio continuo e l'attuazione dei programmi di formazione sono importanti per aumentare la conoscenza delle corrette procedure di pulizia da parte degli operatori e evitare le infezioni.

## INTRODUCTION

Warm water immersion during labour, including birth, used for relaxation and pain relief, has a long history in lay and clinical care<sup>(1)</sup>.

Water births have gradually become more popular in industrialized countries during the last decade. People advocating this form of delivery argue that the buoyancy in water helps the mother to relax and that the warmth helps to reduce pain, meaning that the whole labour process and experience is positively influenced and even accelerated. Due to the sitting position and lower pressure gradient, there are supposedly fewer injuries to the birth canal, and delivery is also claimed to be easier for the child<sup>(2)</sup>.

The positive physiological effects of hydrotherapy such as buoyancy, hydrostatic pressure, and associated thermal changes, are relevant to women labouring in water, where labour is defined as including the first, second (birth) and third stages<sup>(3)</sup>.

Factors such as depth of water, size of the pool and whether the water is still or aerated/whirlpool water have not been compared, as pool design and practice have tended to be based on local availability and customs.

It has been suggested that foetal/neonatal infection may occur due to cross-contamination from the water and pool, and from the woman<sup>(4,5)</sup>. However, several comparative studies, cohort studies, and audits report no increased risk of infection for the foetus/neonate<sup>(6-12)</sup>. As with all maternity provision, it is incumbent upon practitioners to ensure they have appropriate cleaning protocols for labour and birthing pools, and employ universal precautions.

Increased risk to the mother of infection caused by water entering the uterus has been proposed<sup>(13)</sup>.

In "Careggi" Hospital (Florence) there is a big pool called "Daisy" (which has a particular architectural structure that looks like a daisy), specifically built for courses to prepare pregnant women to delivery. Pool parameters (chlorine percentage - accepted range 0.7 - 1.5 mg/l, pH - accepted range 6.5 - 7.5, water temperature - accepted range 32°C - 33°C, environmental temperature - accepted range 28°C - 29°C, humidity - accepted range < or = 70%) are registered all days and should respect the values imposed by the National Health Institute<sup>(14)</sup>. At the end of the activities the bottom of the basin is cleaned through an aspirating system. Once a month (before the beginning of the activities) trained midwives collect water samples for microbiological analysis.

Beyond the big pool, there are several tubs (in single rooms, containing one tub) specifically dedicated to the water birthing. A specific procedure (disposed by the Health Direction) is used to clean the bathtubs and accessories:

- i) the used water should be eliminated and the tub rinsed with high temperature water;
- ii) the disinfectant (with active chlorine) should be used for 15 minutes on the tubs' surfaces and accessories through disposable cloths;
- iii) the bathtub and accessories should be rinsed again with water at high temperature, with pressure jet without forming aerosol;
- iv) further disinfectant should be inserted directly in the tub drain.

The aim of our study was to monitor the effectiveness of the cleaning and disinfecting procedures used for the big pool Daisy and for the tubs for water birthing, also considering the training meetings periodically organized to improve the knowledge about the correct cleaning procedures.

## MATERIALS AND METHODS

We conducted a cross-sectional study in the period January 2016 - July 2017, in the "Daisy" Point of Birth (which has the same name of the "Daisy" big pool) of the Hospital "Careggi" (Florence, Italy).

We collected water samples from the "Daisy" big pool used for pre-delivery courses; we collected also swabs from the tap and from the water discharge system of each used for water tub birthing in conformity with ISO 18593:2004. The microbiological controls were performed monthly (excluding August, because of the unavailability of trained personnel for samplings).

After collection, the samples were marked with an identification code and immediately brought to the Applied Microbiology Laboratory of the Health Science Department of University of Florence, and to Environmental Hygiene Laboratory of the Local Health Unit for the analysis.

Samples were stored at 4°C until testing and all samples were analyzed within 1 h of arrival at the laboratory.

Determination of total bacterial counts at 36°C and 22°C was performed according to UNI EN ISO 6222:2001. Briefly, 1 ml of each water sample was plated in plate count agar and incubated at 36°C +/- 1°C for 48 +/- 4 h and at 22°C +/- 1°C for 64-72 h. Then, plates were read on dark bottom by trained personnel.

Detection and enumeration of intestinal

Enterococci was determined by membrane filtration method according to ISO 7899-2:2003. Briefly, 100 ml of each water sample was filtered on 0.45 micron membrane and then incubated on Slanetz and Bartley agar (Biolife Italiana srl) at 37°C +/- 1°C for 48 +/- 4 h.

Confirmation was carried out in bilesulina agar (Biolife Italiana srl) at 44°C for 24 h: bacteria which were able to reduce 2,3,5-triphenyltetrazolium chloride to formazan and to hydrolyse aesculin on the media were Enterococci.

Enumeration of *E. coli* was carried out by most probable number method according to ISO 9308-2:2012. This method relies upon the detection of *E. coli* based upon expression of the enzyme

D glucuronidase and consequently does not detect many of the enterohaemorrhagic strains of *E. coli*, which do not typically express this enzyme.

Briefly, 100 ml of each water sample was added to the content of one pack in a sterile vessel, according to Manufacturer instruction (Colilert-18/Quanty-Tray, IDEXX, The Netherlands). After dissolving, the mixture was put into a Quanty-Tray and incubated at 35°C +/- 0.5°C for 18 h. Results interpretation included looking the samples for fluorescence with a 365 nm UV light in a dark environment: positive wells become yellow and fluorescent.

The described test provides a confirmed result with no requirement for further confirmation of positive wells.

Enumeration of *Pseudomonas aeruginosa* was determined according to ISTISAN 2007/05 Method ISS A 003A. This method is based on membrane filtration of 250 ml of each water sample and growing on CN-*Pseudomonas* agar (Biolife Italiana srl) at 36°C for 24-48 h. All the fluorescent colonies under a Wood's lamp and with typical smell and also the reddish colonies that didn't fluoresce were considered presumptively as *P. aeruginosa*. The presumptive colonies were confirmed by oxidase test, King's B medium (Biolife Italiana srl) and biochemical identification (Crystal Enteric/Non fermenter, Becton Dickinson, NJ).

Enumeration of coagulase-positive *Staphylococci* was carried out according to ISTISAN 2007/05 Method ISS A 018A. This method is based on membrane filtration of 100 ml of each water sample and growing on Baird Parker agar (Biolife Italiana srl) at 36°C +/- 1°C for 24-48 h. All the presumptive colonies, black shiny and convex with a halo, were confirmed by catalase and coagulase tests (Biolife Italiana srl) and biochemical identification (Crystal Gram Positive, Becton Dickinson, NJ).

Enumeration of moulds and yeasts was

determined according to ISTISAN 2007/05 Method ISS A 016B. Briefly, 100 ml of each water sample was filtered on 0.45 micron membrane and then incubated on Sabouraud Dextrose agar (Biolife Italiana srl) at 20-25°C for 3-5 days. The presumptive colonies were collected and observed on microscopy at 40X and 100X to verify the strain.

The swab technique used allows semi-quantitative analysis because it includes an enrichment phase to resuscitate damaged cells. After the delivery of samples to the laboratory, each swab was immersed in 5.5 ml of Lethen Broth (Thermo Scientific), incubated at 37±1°C for 24 h and then streaked on appropriate culture media, all from Thermo Scientific (Thermo Scientific Oxoid Ltd., Hampshire, UK). *Staphylococcus* spp. detection was obtained on Mannitol Salt agar incubated at 37°C for 24 h. Identification of *S. aureus* suspected colonies was made by means of Api

Staph (bioMérieux Italia Spa, Florence, Italy). Enterobacteriaceae detection was obtained on Desoxycholate agar incubated at 37°C for 24 h. Detection and identification of *Enterococcus* spp. were performed on Slanetz and Bartley agar incubated for 48 h at 44°C. After incubation, suspected colonies of *Enterococcus* spp. were transferred to tryptone soya agar and incubated for 24 h at 37°C and identified through Gram stain, catalase production, and rapid STR (Thermo Scientific). *Pseudomonas* spp. detection was obtained on Cetrimide agar incubated for 48 h at 28°C. Suspected colonies of *P. aeruginosa* were identified through Microbact 24E (Thermo Scientific).

Yeast and molds detection was obtained on Rose Bengal agar incubated for 48 h at 28°C and through optical microscopy observations.

Semi-quantitative results were expressed as follows: - contamination not detected; + low contamination; ++ medium contamination; +++ high contamination.

The results were organized in a database and then exported for statistical analysis. Percentages, means and standard deviations were calculated, followed by the creation of tables for a descriptive purpose. Fisher's exact test, calculation of Odds Ratios and Mann Whitney Test were performed in order to identify significant differences. The non normal distribution of the examined variables was assessed through the Shapiro-Wilk test. The collected data were organized and processed using the software Stata® SE, version 12.1 (StataCorp, College Station, Texas, USA). The level of significance was set at  $p < 0.05$

## RESULTS

From January 2016 to July 2017 we collected 15 water samples from the big pool of the “Daisy” Point of Birth: 9 (60.0%) in 2016, 6 (40.0%) in 2017. *E. coli*, *Pseudomonas aeruginosa* and *Enterococcus spp.* resulted negative in all the samples.

2 samples (13.33%) resulted positive for 36°C CFU (mean CFU 106.5, SD 146.37), 2 samples (13.33%) resulted positive for 22°C CFU (mean CFU 56.5, SD 75.66).

Mann Whitney test showed that there wasn’t a statistically significant difference both in the CFU at 36°C and at 22°C among the two analysed years (all  $p > 0.05$ ). (Figure 1)

From January 2016 to July 2017 we collected 142 samples from the 4 tubs for the birth in water. 88 (61.97%) were collected in 2016, 54 (38.03%) in 2017. The buffers were rubbed to the tap and to the water discharge.

The positive samples for each detected microorganism are resumed in tables: **Table 1**, **Table 2**.

There was a statistically significant difference ( $p = 0.01$ ; OR=0.28) in the presence of staphylococci in the two examined years: in 2017 their presence was significantly lower (N=5) then 2016 (N=23).

There was also a statistically significant difference ( $p = 0.03$ ) in the presence of yeasts in the

Figure 1. Temporal trend of the 36°C and 22°C CFU in the examined period of time

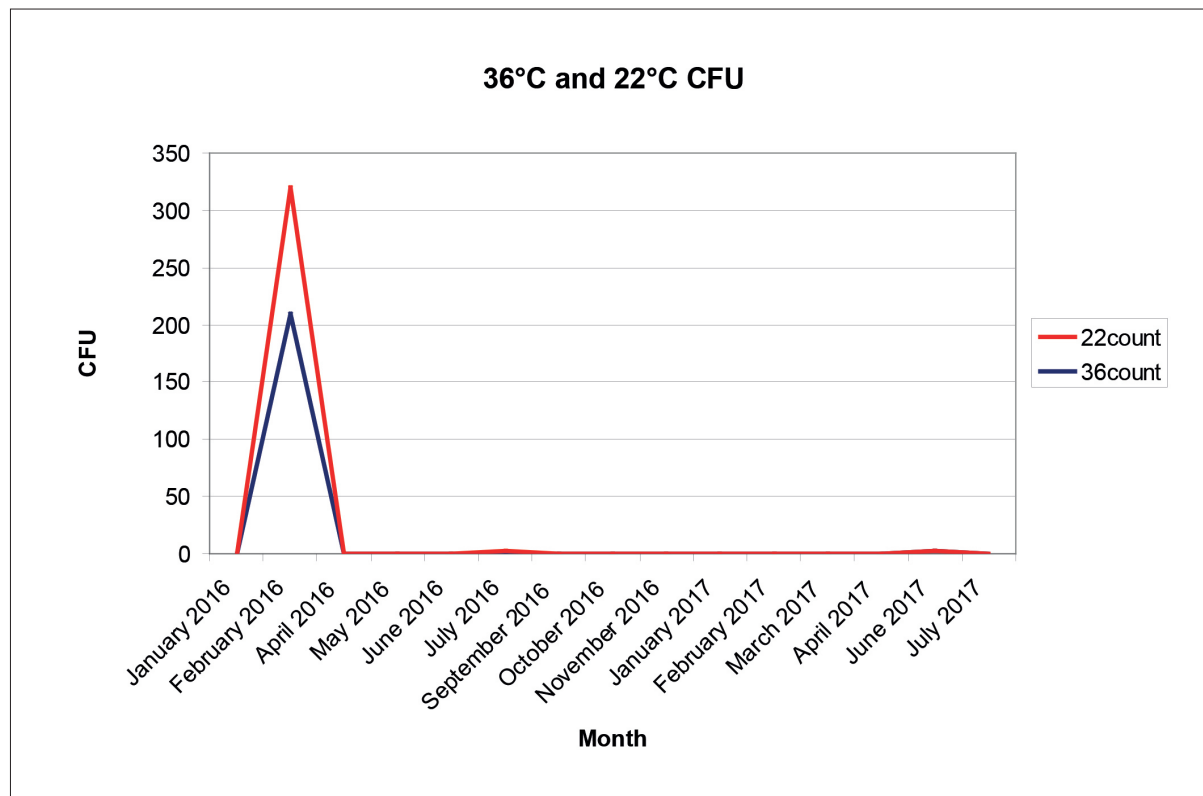


Table 1. Positive samples for each microorganism (\* percentage calculated on 142 samples)

	Frequency (N)	Percentage*
<i>Staphylococcus spp</i>	28	19.72
<i>Enterobacteriaceae</i>	3	2.11
<i>Yeasts, Moulds</i>	6	4.23
<i>Pseudomonas spp</i>	0	0
<i>Enterococcus spp</i>	0	0



**Table 2.** Positive samples by level of contamination (low, medium, high) and year

	2016			2017		
	Low	Medium	High	Low	Medium	High
<i>Staphylococcus spp</i>	8	6	9	0	2	3
<i>Enterobacteriaceae</i>	2	0	1	0	0	0
<i>Yeasts, Moulds</i>	0	0	0	6	0	0
<i>Pseudomonas spp</i>	0	0	0	0	0	0
<i>Enterococcus spp</i>	0	0	0	0	0	0

two examined years: in 2016 their presence was null, in 2017 six samples resulted positive.

Chi square test evidenced also that there wasn't a statistically significant difference in the positivity among the rooms (all  $p > 0.05$ ), but a significant difference due to the site of sampling. Water discharge system was at a higher risk of being contaminated by staphylococci ( $p = 0.01$ , OR=3.08).

## DISCUSSION

As several studies demonstrated, pregnant women exercise in the water offers several physiological advantages: the hydrostatic force of water pushes extravascular fluid into the vascular spaces, producing an increase in central blood volume that may lead to increased uterine blood flow. This force is proportional to the depth of immersion. Moreover, the increase in blood volume is proportional to the woman's oedema. A marked diuresis and natriuresis accompanies the fluid shifts. The buoyancy of water supports the pregnant women. Water is thermoregulating. Pregnant women's heart rates and blood pressures during water exercise are lower than on land exercise, reflecting the immersion-induced increase in circulating blood volume. The physiology of water exercise offers some compensation for the physiological changes of exercise on land that may beneficially affect pregnancy<sup>(15)</sup>.

However, it is known also that swimming pools can be the source of infections due to micro-organism but effective preventive measures (including the continuous recording of the redox-potential of the water, limiting the number of visitors to pool, a better disinfection of sanitary installations, regular maintenance of technical equipment including frequent backwashing of filters and exclusion of visitors with communicable disease) could avoid their transmission<sup>(16)</sup>.

Since the chemical-physical characteristics of water can affect the degree of attachment of microorganisms to a substrate, the first step for the

formation of biofilms, and the water can constitute up to 97% of the biofilm, it can be assumed that technologies capable of altering the physical state of water can change a suitable setting for microbial growth into an unsuitable one; in addition, it may also modify the matrix of the biofilm by acting on its most representative constituent or by changing the interaction that this matrix has with the surrounding aqueous environment<sup>(17,18)</sup>.

A right education of healthcare providers and the control of applications following training are very important in the prevention of nosocomial infections<sup>(19)</sup>.

In fact, as we can see from our results there is a remarkable difference between 2016 and 2017 in the microbiological results both for the big pool and for the tubs: these results could be explained with the training meetings that we decided to organize because of the results obtained in 2016.

After the non-normal results (within 1 or 2 days), representatives of the cleaning company, and of the Daisy Point of Birth, were summoned in order to understand the reasons of these results and to detect strategies to avoid them. The critical points that were detected were: the operators (so it was decided to use the same trained operators to clean the critical areas, in order to avoid a operator-dependent bias), the type of cleaning (so the operators were trained to deeply clean also the inner part of tap, and the bottom of the basin to avoid a retrograde contamination), and the time of sample collection (collection should be performed immediately after the cleaning). In order to confirm the efficacy of these meetings (and of the following training meetings that each representative made with the operators showing them the critical points and the identified solutions) further samplings were performed (all showing normal results - all negative).

In the tubs we found, in 2016, positivity for staphylococci and enterobacteria; in 2017 for yeasts, but with low contamination. No samples resulted positive for *Pseudomonas* and *Enterococci*. This result is quite different from

what reported by Thoni et al. in 2007: in their study, collected samples contained *Legionella* in 29%, *Pseudomonas aeruginosa* in 22%, enterococci in 18%, colibacilli in 32% and *Escherichia coli* in 8%. After fitting a filter system, no *Legionella* was detected any more. *P. aeruginosa* was found in only 3% of the samples<sup>(20)</sup>.

The risk represented by the positivity for bacteria, and especially enterococci and staphylococci, is very high: Rawal et al. in 1994 examined the hazards associated with births in water. They have reported the case of a baby who became colonised with a virulent organism during water birth. The blood cultures were sterile, which argues against him being frankly septicaemic. Nevertheless, he behaved and looked like a septic baby and he responded convincingly to antibiotics. This case highlighted an important potential hazard of water birth<sup>(21)</sup>. In 1994, also Coombs et al. reviewed infection in the babies born to mothers who had used pools in labour. From December 1991, 122 mothers used the pool in labour, of whom 41 delivered in the pool. They swabbed the ears of all infants whose mothers have used the pool and recorded their respiratory rate for three hours after delivery. Three of the 122 babies had positive ear swabs. Two of them were well with their mothers on the postnatal wards. The swabs grew group B streptococci and *Staphylococcus aureus*, and both babies were treated with antibiotics until further cultures were negative. The last baby presented at 10 days with greenish discharge from his ear. Five of the 122 infants were admitted to the special care baby unit with a raised respiratory rate or grunting. Four of the five had been delivered under water. All were treated with antibiotics, though in all swabs and blood cultures were negative<sup>(22)</sup>.

However the discussion about the real connection between birth in water and infection is still open: a recent systematic review (2014) reported that there are no differences in infection rates and admissions to neonatal intensive care

due to this procedure. The authors concluded that water births appeared to be associated with minimal risk, and the maternal and neonatal outcomes were similar to those of healthy childbirth populations<sup>(23)</sup>.

The reduction of the incidence of healthcare-associated infections requires proper environmental cleanliness and a right and continuous education and training of nursing and environmental services staff to reduce healthcare-associated infections. In Cook Children's Medical Center during the study period, it was observed a significant decline of nosocomial infections<sup>(24)</sup>. Although no cases of infections associated with the birth in water were registered in our Hospital, some positive samples observed in some periods of 2016 obliged us to improve and implement the knowledge about the right sanitizing technique of the operators and led to a significant reduction of these positive samples. The higher contamination observed in the discharge system of the tubs was solved inserting chlorine-based tablets directly in the discharge system.

As observed by Demitürk et al in 2006, the level of knowledge regarding nosocomial infections and their prevention among previously untrained cleaning staff is quite low. The level of knowledge among previously trained participants instead was higher. These results suggested that training of the cleaning staff of the hospital as well as of healthcare professionals are key factors in prevention of nosocomial infections<sup>(25)</sup>.

One of the most important limits of our study was represented by the lack of information about some months of the two years (essentially represented by summer months), due to the summer holidays. However, the long period of observation and the training courses periodically organized let us have a wide picture of the microbiological contamination of the Daisy Point of Birth which could be considered an indirect method to assess the quality of cleaning and of operator's knowledge.

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