DETECTION OF THE PRESENCE OF PSEUDOMONAS AERUGINOSA AND CONTROL MEASURES IN THERMAL WATERS

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ABSTRACT

Thermal waters in Colombia are not tested for quality to ensure users health and their aesthetic quality because of the high costs of these procedures. This study shows the results of water tests performed in three thermal water pools in Cundinamarca, randomly chosen to estimate the presence of *Pseudomonas aeruginosa*; these tests are not statistical due to the available resources. Results were compared with international standards because Colombian regulations have yet to be passed as laws. The results showed the presence of *Pseudomonas aeruginosa* in one of the analyzed thermal water sources. As a hypothesis, it was established that this is a consequence of an inadequate protection of aquifer or waste discharge in the thermal water spring. Besides, the concentration of *Pseudomonas aeruginosa* in wells and pools was found to be under 1 UFC/100 ml for pH values under 7.00. While for pH values above 7.00, the presence of the pathogen can be counted, which is why control measures were formulated to keep in line the levels of contamination of thermal water pools; these measures comprise medical, hygiene, and usage restrictions.

Keywords:
Colombia, *Pseudomonas aeruginosa*; Thermal water, Pseudalert, Regulations, Control measures

INTRODUCTION

Thermal waters in Colombia are used for recreational, therapeutic, and medicinal purposes. Health benefits are attached to them by improving blood pressure, oxygenation, and being used to treat rheumatoid arthritis, breathing, and skin conditions. The regulations for using and taking advantage of thermal waters is the bill draft, number 62 of 2015, which aims at promoting, fostering, regulating, and controlling the therapeutic and touristic usage of thermal spas and the use of thermal

waters. However, it does not make any reference to water quality at the microbiological level, which makes it an unexplored field in Colombia. (Chivatá 2016).

This study shows a random sampling from three thermal water pools in Cundinamarca, Colombia (Tabio, Guasca, and Choachi), to detect the presence or absence of *Pseudomonas aeruginosa* which can survive elevated temperatures and with a shallow nutritional need. (Chivatá 2016). This pathogen can cause folliculitis and swimmer's ear (Centers for Disease Control and Prevention - U.S. Department of Health and Human Services. (n.d.)); it can also bring about health issues for cancer, immunologic, and immunocompetent patients.

The article *Antimicrobial resistance in Pseudomonas aeruginosa strains in thermal waters of the Province of Chimborazo, Ecuador* states that: The presence of *Pseudomonas aeruginosa* in thermal water may reflect a significant contamination of it. An explanation for this fact may be; the contamination of the mineral water source or spring, the lack of adequate aquifer protection, biofilms presence on the walls or rocks from where the water comes out, runoff waters, or animal and human waste discharge in the thermal springs (Andueza, et al 2015).

This study aims to prove the presence of *Pseudomonas aeruginosa* in both thermal water sources and pools, to determine control measures that help ameliorate the presence of this pathogen in thermal waters. For this purpose, research was pursued regarding national and international regulations for the presence of *Pseudomonas aeruginosa*, as well as quality standards that thermal water spas should comply with in order to offer this service. In the legal framework, a summary of international regulations was made, based on countries such as Spain, Chile, Cuba, and the ones by the World Health Organization (Chivatá 2016).

METHODS

For developing this study, 18 samples from three public use thermal water pools were analyzed, measuring pH, temperature, and *Pseudomonas aeruginosa*. These pools are in Tabio, Guasca, and Choachí, municipalities of the department of Cundinamarca (Colombia). The pools were selected based on the following factors:

1. Lower transportation time from site to lab, due to the transportation costs of samples (Chivatá 2016).
2. There are five thermal blocks in Colombia, and the study was performed in thermal block number 1, which is comprises of the departments of Cundinamarca, Santander, and Norte de Santander (INGEOMINAS, 2003).
3. Public use adult thermal waters, due to a higher number of users (Chivatá 2016).

The sampling interval for each thermal water pool was two weeks during a one and a half months' period during vacation season (June and July of 2016), when most swimmers attend these places. In situ, physical measurements were performed as described in **Table 1** and lab measurements in

Table 2 based on the Standard Methods for the Examination of Water and Wastewater, 2005.

Table 1. Physical parameters measured *in situ*

Parameter	Unit	Tool	Analysis method
Temperature	°C	pH-metro 3110	SM, 2550 B
pH	pH unit	pH-metro 3110	Electrometrical, SM 4500-H+ B.

Source: (Chivatá 2016).

Table 2. Microbiological parameters measured in lab

Parameter	Unit	Method	Cultivation method	Incubation period
Pseudomonas aeruginosa	NMP/100 ml	Defined substrate, SM 9213 F Modified	Pseudalert	38.5 °C for 24 hours

Source: (Chivatá 2016).

Pseudomonas aeruginosa was detected through *Defined Substrate Technology* (DST), which is based on a bacterial enzyme detection technology that shows the presence of such pathogen through substrate hydrolysis in the Pseudalert reactive compound. *Pseudomonas aeruginosa* cells grow and multiply rapidly due to a significant amount of amino acids, vitamins, and other nutrients present in the reactive compound. *Pseudomonas aeruginosa* strains that are actively growing have an enzyme that hydrolyzes the reactive substrate emitting a blue fluorescent light when exposed to UV light (IDEXX Laboratories, n.d.).

Defined Substrate Technology (DST) is based on the Standard Methods Technique 9213F of multiple tubes for *Pseudomonas aeruginosa*, since it is an improved technology to measure this pathogen, which is performed according to the following procedure (IDEXX Laboratories, n.d.):

1. The Pseudalert diagnosis reactive is added to 100 ml thermal water, and the samples are grown in a laminar flow cabinet.
2. The mixture is poured into Quantitray bags and sealed.
3. Bags are incubated for 24 hours at 38.5°C.
4. Positive wells count is performed using a 6 watt fluorescent U.V. lamp held 12 cm away from the sample.
5. After the procedure, wells with a blue fluorescent light indicate the presence of *Pseudomonas aeruginosa*.

The microbiological results were assessed based on the established standards for spas issued by the World Health Organization (WHO) and the regulations issued by Cuba and the autonomous communities of Galicia, Murcia, and Catalonia in Spain, due to the lack of standards in Colombia. The established ranges are shown below (see **Table 3).**

Table 3. International regulations for thermal waters

ZONA	SPAIN			CUBA	WHO
	Galicia	**Catalonia**	**Murcia**	**State**	**World**
REGULATIONS	Order, November 5, 1996 (Xunta of Galicia,1996)	Decree 271 of 2001, October 9 *(Sintesi)*	Decree 55 July 11 of 1997 (Ministry of Health and Social Policy)	NC 93-09:85 NC 93-28:88 (Ministry of Public Health, 2002)	Guidelines for safe recreational water environments Volume 2 2006 (World Health Organization, 2006)
PARAMETERS					
***Pseudomonas aeruginosa* (NMP/100 ml)**	Free from pathogenic microorganisms and parasites	Free from pathogenic microorganisms	Absence in 1 g or 100 ml	< 2.2 NMP/100 ml	Weekly <10 UFC/100 ml

Source: (Chivatá 2016).

RESULTS AND DISCUSSION

Physical tests

Temperature. Analyzed thermal water sources had temperatures between 38 and 50 °C, which classify them as mesothermal waters by being within the 35 to 50 °C range, closing into a hyperthermal condition. (See **¡Error! No se encuentra el origen de la referencia.**).

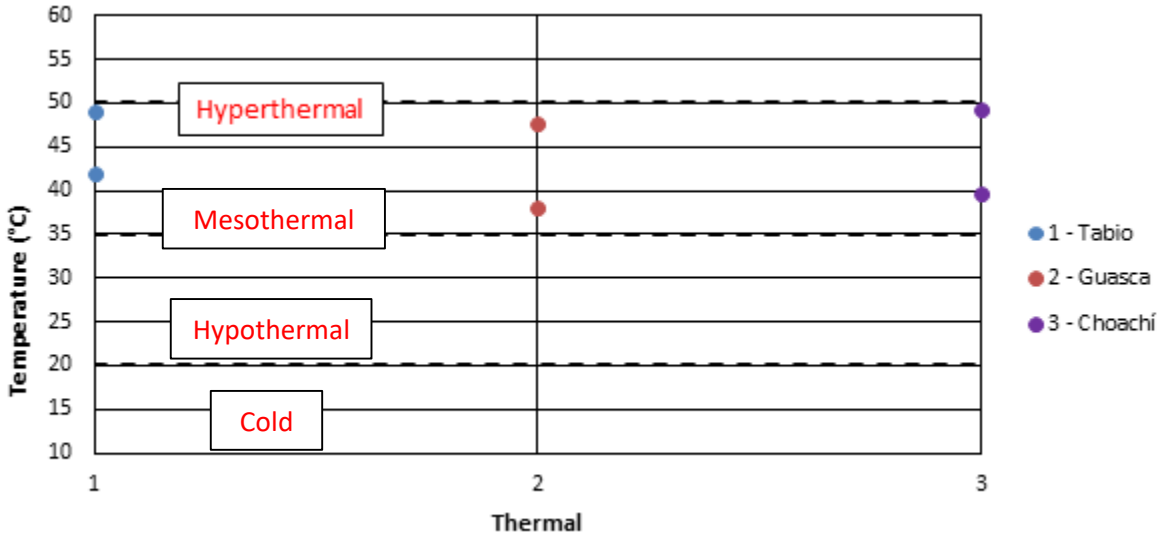

Figure 1. Analyzed thermal water sources temperature.
Source: (Chivatá 2016).

pH. pH in analyzed thermal water sources was in the 6.0 to 7.10 range and within a 6.61 to 7.70 range for pools, which place them in like acid and alkaline thermal waters. (See Figure 2 and Figure 3).

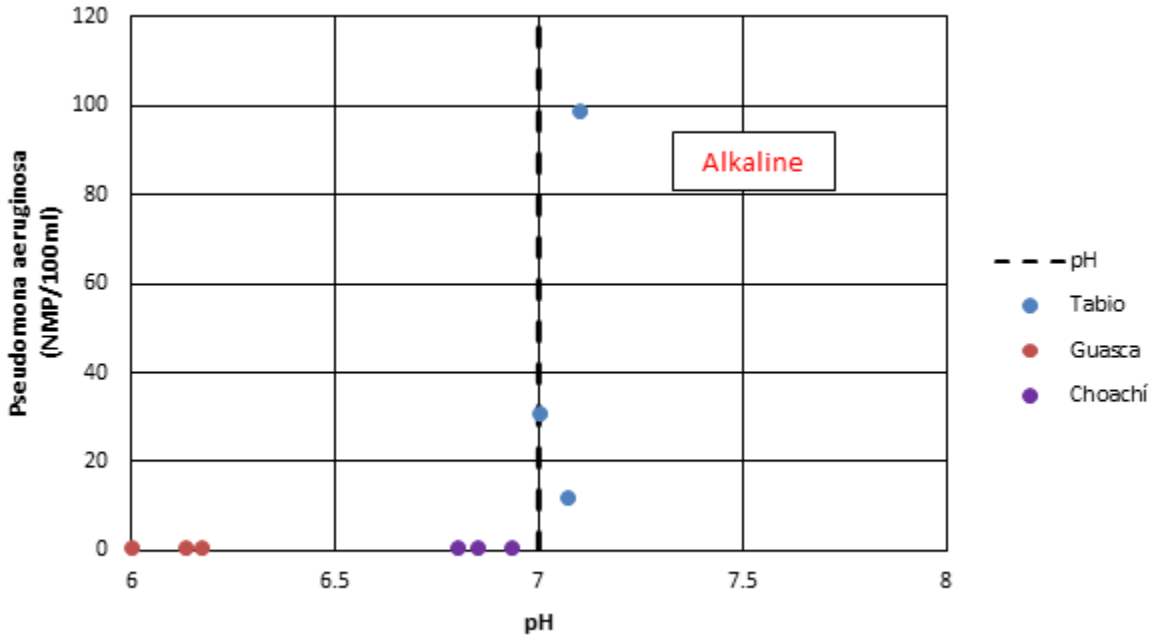

Figure 2. *Pseudomonas aeruginosa* in the thermal water well.
Source: (Chivatá 2016).

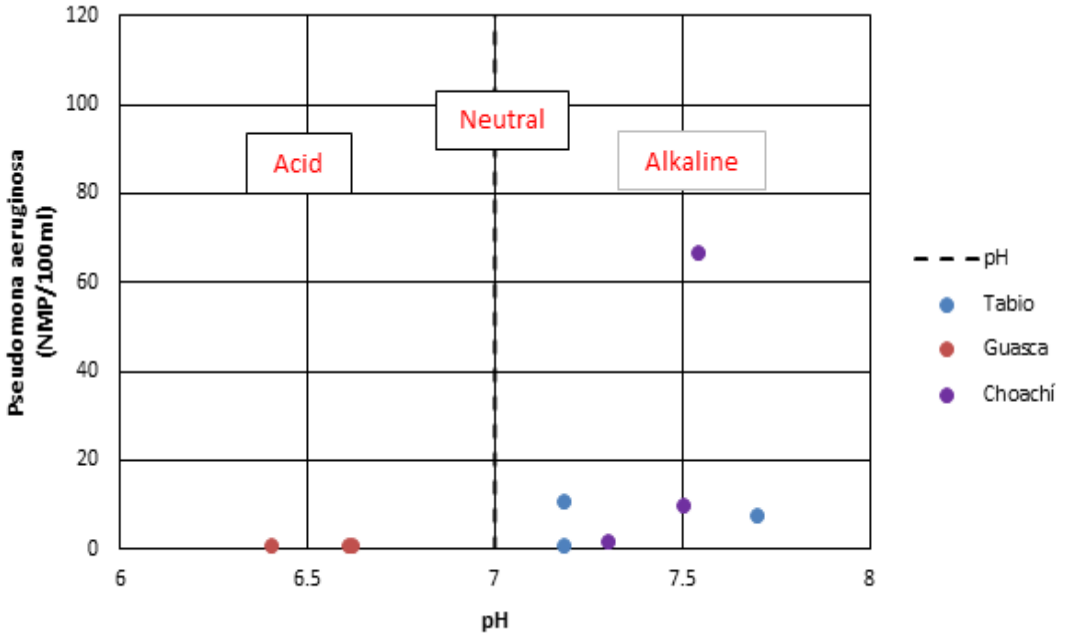

Figure 3. ***Pseudomonas aeruginosa*** **in thermal water pools.**
Source: (Chivatá 2016).

Microbiological trials

Pseudomonas aeruginosa. Microbiological tests showed the presence of *Pseudomonas aeruginosa* in 1 out of 3 analyzed wells and 2 out of 3 analyzed pools.

The presence of the pathogen in the well may indicate poor protection of the source spring, although it can colonize aquatic environments and be found in underground waters uncontaminated by man (De la Rosa Jorge & Mosso Romeo, 2000).

In those pools where the pathogen was found, the concentration varies between 2 UFC/100 ml and 67 UFC/100 ml and the one in the well ranges from 12 UFC/100 ml and 99 UFC/100 ml.

Correlation between pH and Pseudomonas aeruginosa

Guasca's thermals, whose wells pH variation is from 6.00 to 6.17 and the pools are from 6.40 to 6.62, displayed a pathogen concentration lower than 1 UFC/100 ml.

Choachí's thermal wells pH variation is between 6.80 and 6.93, the pathogen's concentration is lower than 1 UFC/100 ml, the pool's pH varies from 7.30 to 7.54, and the pathogen's concentration is between 2 UFC/100 ml and 67 UFC/100 ml.

Lastly, at Tabio's thermals, the wells pH variation was from 7.00 to 7.10 there is a pathogen's concentration between 12 UFC/100 ml and 99 UFC/100 ml, the pool's pH varies between 7.18 and 7.70 and the pathogen's concentration was found to vary between 1 UFC/100 ml and 11 UFC/100 ml.

Based on the results obtained from the analyzed samples at Tabio, Guasca, and Choachí's thermals, it could be observed that both in pools and wells, the presence of *Pseudomonas aeruginosa* accentuates when the substance is classified as alkaline, that is, pH values higher than 7.0 (See Figure **2** and Figure **3**).

The association between water pH and the pathogen presence agrees with the conclusions of the study named "Microbial diversity of mineral thermal waters." Where the following is stated: "Alkaline pH springs have low microbial diversity, finding Pseudomonas, Bacillus, and Exiguobacterium that can survive these pH levels and acid pH 70 show a minimal number of bacteria, mainly Gram-positive irregular bacilli.

Pseudomonas aeruginosa and thermal waters regulations

As a reference, the ranges established for *Pseudomonas aeruginosa* from standards issued by Cuba, WHO, Galicia, Cataluña, and Murcia in Spain (**Table 3**), an assessment of the results found in wells and pools discovered in this study is presented (**Tables 4 and 5**).

Table 4. Well results assessed according to international regulations for thermal waters

COUNTRY							
ZONE			**SPAIN**			**CUBA**	**WHO**
			Galicia	**Catalonia**	**Murcia**	**State**	**Mundial**
PARÁMETROS							
***Pseudomonas aeruginosa* (NMP/100 ml)**			Free from pathogenic microorganisms and parasites	Free from pathogenic microorganisms	Absence in 1 g or 100 ml	< 2.2 NMP/100 ml	Weekly <10 UFC/100 ml
THERMAL	**CAMPAIGN**	**P.A. (NMP/100 ml)**	**WELL RESULTS**				
Tabio	1	31	Uncompliant	Uncompliant	Uncompliant	Uncompliant	Uncompliant
	2	12					
	3	99					
Guasca	1	< 1	Compliant	Compliant	Compliant	Compliant	Compliant
	2	< 1					
	3	< 1					
Choachí	1	< 1	Compliant	Compliant	Compliant	Compliant	Compliant

	2	< 1					
	3	< 1					

COUNTRY							
ZONE			SPAIN			CUBA	WHO
			Galicia	Catalonia	Murcia	State	World
PARAMETERS							
***Pseudomonas aeruginosa* (NMP/100 ml)**			Free from pathogenic microorganisms and parasites	Free from pathogenic microorganisms	Absence in 1 g or 100 ml	< 2.2 NMP/100 ml	Weekly <10 UFC/100 ml
THERMAL	**CAMPAIGN**	**P.A. (NMP/100ml)**	**POOL RESULTS**				
Tabio	1	8	Uncompliant	Uncompliant	Uncompliant	Uncompliant	Compliant
	2	< 1	Compliant	Compliant	Compliant	Compliant	Compliant
	3	11	Uncompliant	Uncompliant	Uncompliant	Uncompliant	Uncompliant
Guasca	1	< 1	Compliant	Compliant	Compliant	Compliant	Compliant
	2	< 1	Compliant				
	3	< 1	Compliant				
Choachí	1	2	Uncompliant	Uncompliant	Uncompliant	Compliant	Compliant
	2	10				Uncompliant	Uncompliant
	3	67					

Source: (Chivatá 2016).

Table 5. Pool results assessed with international regulations for thermal waters

Source: (Chivatá 2016).

Swimmers and determination of control measures

Several polluting agents of water exist. These can come from previous water contamination, poor or lack of cleaning of the pool and its surroundings, the accessories immersed, and **mainly users themselves**. Each swimmer can bring a significant amount of mineral and organic matter into the pool, along with millions of saprophyte germs, or even pathogens from different origins, such as ear and nose, genital and urinary, digestive, and cutaneous (Bacaicoa n.d).

To prevent contamination in the water and the surroundings of the treatment pools, as well as fighting the development of infectious agents, it is necessary to fully comply with health and cleaning guidelines for swimmers, pools, and every single facility. For users of treatment pools,

top body hygiene be required, such requirement must be demanded by health personnel to favor health education (Bacaicoa n.d.).

The following suggestions, among others, are given as control measures for thermal water pool users:

- A physician must assess every person before entering (Bacaicoa n.d.).
- No entry allowed for pregnant women, people with skin conditions, burns, open wounds, or those with immunosuppressive diseases (Bacaicoa n.d.).
- Every user must take a thorough shower before entering (Bacaicoa n.d.).
- Every person should use the foot shower before and after entering thermal pool. *(*Bacaicoa n.d.).
- Appropriate swimwear and swimming cap must be used (Bacaicoa n.d.).
- No entry allowed for people using t-shirts, shorts or any other type of clothing (Bacaicoa n.d.).
- No food or drinks allowed in the pool and its surroundings (rules in the visited spas).
- No smoking, spitting or pets allowed (Bacaicoa n.d.).
- Swimmers must take off their swimwear as soon as possible after exiting the pool (Centers for Disease Control and Prevention - U.S. Department of Health and Human Services).
- Swimwear must be washed right after exiting the pool (Centers for Disease Control and Prevention - U.S. Department of Health and Human Services).

For thermal water pools, extreme health and cleaning habits are suggested, such as:

- To clean the pool, it must be emptied entirely (Bacaicoa n.d.).
- Pool walls must be scrubbed thoroughly with brooms, brushes, detergents or grease removers, and washed with plenty of water (maintenance actions performed in the visited spas).
- All facilities inside the spa (except the pool), such as floors, showers, dressing rooms, toilets, foot showers, and others must be disinfected daily.

In places utilized for thermal water enjoyment, deep cleaning and maintenance activities are expected to be performed to guarantee thermal water quality, due to the therapeutic and medicinal benefits attributed to this type of water.

For successful controls, spa managers should raise awareness among users about the importance of stating their actual health state to provide the best possible service and to watch over their health and safety.

Nowadays in Colombia, proposed control measures are inspired by public swimming pool legislation, but not for thermal water pools. Thus, it is necessary to regulate the microbiological quality of thermal water pools, specifying correct regulations for thermal water spas to keep the bacteriological and chemical quality of mineral waters without substantially affecting mineral medicinal waters.

In France, thermal spas can have a 10^2 UFC/L value for *Pseudomonas aeruginosa* as a reference value; above this value, special attention must be paid. To comply with this, special measures have been taken to fight its proliferation, such as daily disinfection of almost all the thermal water distribution network which, according to GARANS, a sanitary engineer from Marseille, is the only procedure that can significantly bring down the presence rate of *Pseudomonas aeruginosa* and *Legionella*. This procedure proved in a six-month period, through 182 official analyses and 665 self-control analyses, an elimination of this type of contaminant (Bacaicoa, n.d.)

Another aspect that proved to be relevant to favor water quality is the attention needed to obtain a complete water renovation, by avoiding still waters these dead zones can become up to 50% of pool flow and are favored by accessory material and especially the number of swimmers. Most of the renovation water is poorly used if dead zones exist. Favorable conditions for water renovation can limit and even avoid the usage of disinfecting products for pools, according to COLLIN, to keep an acceptable microbiological quality, by keeping the thermal water unmodified. However this issue is still being argued at international scientific events about Hydrology or Thermalism (Bacaicoa, n.d.).

CONCLUSIONS

- *Pseudomonas aeruginosa* presence is observed in one out of three analyzed thermal water wells. As a hypothesis, it is established that such contamination can be associated with an inadequate protection of the aquifer or due to waste discharge into the thermal water spring.
- *Pseudomonas aeruginosa* concentration, both in wells and pools for pH levels under 7.00 (acid) is below 1 UFC/100 ml, while for pH levels above 7.00 (alkaline) the pathogen's presence becomes countable.
- 67 % of sampled thermal water from supply wells comply with the established ranges issued by consulted regulations, while only 56% of sampled water from pools met the requirements.
- Overall, out of eighteen analyzed samples (taken from wells and pools), only 61% of them met the consulted regulations.
- To control contamination levels resulting from its usage, it is necessary to implement a controlled entry system that considers medical, hygiene, and usage restrictions.

- Because of cultural reasons, people in Colombia who go to thermal pools usually have a disease and visit these spas for their medicinal attributes, which make them more vulnerable to *Pseudomonas aeruginosa* related infections
- Colombia does not have any regulations for thermal waters, control measures based on public pools legislation are proposed, but these measures are not for thermal water pools. It is necessary to regulate the microbiological quality of thermal water pools, specifying applicable rules for thermal water spas to keep mineral waters' bacteriological and chemical quality without affecting mineral medicinal waters substantially.

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