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Hydrogen sulfide in thermal spring waters and its action on bacteria of human origin

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ABSTRACT

Hydrogen sulfide (H_2S) is a molecule dissolved in many thermal spring waters at variable concentration. The H_2S effects of thermal waters treatments have long been studied, for dermatological and clinical treatments, but its role in recreational waters was never investigated. The use of sulfur spring waters in pools raises concerns related to disinfection by oxidants. The aim of this work is to evaluate the survival rate of microbial species in waters with different titers of H_2S. Four selected thermal waters collected from Italian springs, belonging to different chemical categories, have been tested in comparison to Tyrrhenian sea water and natural mineral bottled water. Results show inhibition properties on bacterial proliferation that seem related to H_2S concentrations. To further asses this phenomenon H_2S was added to thermal and natural mineral waters. The results strongly support a bactericidal role of H_2S in thermal spring waters used for recreational purposes. These observations open up new perspectives for a disinfectant role of H_2S in pool treatment and management.

1. Introduction

Hydrogen sulfide (H_2S) is a colorless, flammable gas with a "rotten egg" smell. It is one of the principal components in the natural sulfur cycle. Bacteria and fungi release H_2S during the decomposition of sulfur-containing proteins or by the direct reduction of sulfate (SO_4^{2-}). H_2S is also consumed by bacteria found in soil and water by the oxidation of hydrogen sulfide to elemental sulfur. Photosynthetic bacteria can oxidize H_2S to sulfur and sulfate in the presence of light and the absence of oxygen [1,2]. H_2S is commonly emitted from volcanoes, stagnant or polluted waters, hot springs, and underwater thermal vents. These natural sources account for about 90% of the total hydrogen sulfide in the atmosphere. Although H_2S has been studied for its toxic properties, several recent experimental evidences suggest that H_2S may play a prominent role in normal physiology and pathophysiology. Therefore, many therapeutic targets exist for H_2S therapy, including cancer, heart failure, organ transplant, peripheral artery disease, inflammatory bowel disease, Alzheimer's disease, acute myocardial infarction (MI), stroke, atherosclerosis, hypertension, erectile dysfunction, metabolic syndrome, diabetes, and thrombosis [3–8].

H_2S is dissolved in many thermal spring waters at variable concentration, used by centuries in different countries [9]. In general, spring waters have different chemical compositions, which can vary considerably depending on the location of the source (e.g. near the sea, volcanic, geothermic or mountain areas, etc.). The thermal waters are described as salty, sulfurous, bicarbonate, sulfated, carbonic, arsenical, or ferruginous, on the basis of their mineral composition [10]. The chemical concentration of thermal water is directly correlated to its therapeutic effects. To date, the chemical elements and mechanisms involved in the health benefits of hot springs remain still unclear [11].

Several authors reported bactericidal and antifungal activity from thermal spring waters with special focus on dermatological diseases [11–14]. Large importance has been dedicated to the pH level or to the presence of specific cations or anions in the water. The H_2S may be present under certain conditions as different reactive sulfur species. For example, the interaction between sulfur and oxygen radicals leads to the formation of subproducts, such as pentathionic acid ($H_2S_5O_6$), which may represent the source of the antibacterial and antifungal activity of thermal water on the skin [12–15]. This specific bactericidal activity can be particularly important in the management of thermal waters for recreational use [16]. Pools used for thermal baths in "salus per aquam" resorts (SPA) may be characterized by bacteria contamination due to overcrowding pool conditions. The microbiological risk is commonly countered by disinfection procedures. Chlorination or ozone treatments, largely adopted in conventional swimming pools, are not applicable to SPA pools or swimming ponds [17], due to the high level of reducing agents and salts present in natural thermal waters. Thus oxidative methods should not be applied in thermal waters as they may alter chemical components and,

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consequently modify beneficial effects and determine plant management problems due to the induced chemical reactions and salt precipitation [16].

In consideration that the dissociation of H_2S is strongly dependent on the pH value, and that the effect of waters with neutral pH but variable H_2S concentrations on microorganisms has not been investigated, an analysis of human-born bacteria survival in four Italian thermal spring waters has been conducted. The selected waters showed pH values between 6 and 8 and a strong variability in H_2S concentration. Artificial addition of H_2S to thermal and not thermal waters was also performed to assess its role in decreasing bacterial load.

2. Material and methods

2.1. Water sampling and analysis

Within a survey of Italian spas' waters, four thermal springs with significant differences in chemical composition have been selected. The thermal water samples were collected in sterile glass bottles of 1 L, filled and immediately hermetically sealed to prevent release of gases. During the sampling activities, temperature, pH and H_2S were determined.

One sample of Tyrrhenian sea costal water was collected in the same way; the sample was then filtered through a 0.22 μm membrane in cellulose acetate (Whatman-GE Healthcare, USA).

A commercially available bottled natural mineral water (BNM) has been included in the experiments as reference with a low salt content and microbiologically safe. The fixed residue at 180 °C was 0.3 g/L, while the pH value was equal to 7.2.

Chemical composition of the four thermal waters was determined by different analytical procedures: sodium was quantified by an Italian official method [18] based on the inductively coupled plasma optical emission spectrometry (ICP-OES); bromide and iodide were quantified by an Italian official method [19] based on the ionic chromatography; H_2S was quantified by an Italian official method [20] based on iodimetric titration; pH was measured by the Italian official potentiometric method [21]; fixed residue was determined by the Italian official gravimetric method by using platinum capsules [22].

2.2. Generation of H_2S and bubbling in water

A stream of H_2S was obtained from a Kipp apparatus, where FeS (C. Erba RPE p.a.) was treated with 25% HCl. Hydrochloric acid was obtained by diluting a C. Erba RPE p.a. reagent with water prepared by passing distilled water through a 4-cartridge Millipore Q water purification system.

To avoid the possibility that traces of HCl could be carried away with the stream of H_2S, a flock of glass wool and a Drexel with distilled water were inserted between the Kipp device and the test solution.

The absence of hydrochloric acid in the sample was further checked by analyzing the distilled water of the Drexel. A measured volume of the water was boiled during 20 min to eliminate the presence of H_2S and to concentrate the solution. After cooling, HNO_3 and 1.0 mol dm^{-3} $AgNO_3$ were added to have the concentration of $Ag^+ \approx 0.01$ mol dm^{-3}. As no cloudiness was observed, Cl^- concentration was $\leq 10^{-8}$ mol dm^{-3}.

The test solutions were analyzed before and after the bubbling of H_2S. All measurements were carried out at room temperature (20 °C).

The pH value of the solutions was measured before and after the addition of H_2S. In both cases, the measure was performed potentiometrically [21].

The concentration of H_2S in the test solutions was determined by titration [20].

2.3. Microbiological tests

Classical pool indicators of microbial contamination were considered, following WHO guidelines indications [16]. In particular *Escherichia coli* ATCC 35218, *E. faecalis* ATCC 7080, *Staphylococcus aureus* ATCC 6538 strains were used in this study. The stock culture, stored at −20 °C was transferred in TSA agar plates (Tryptone soya agar, OXOID, Cambridge, UK). After 18 h of incubation at 37 °C, one colony was picked and inoculated into a 15 mL sterile tube containing 3 mL of TSB (Tryptic Soy Broth, Difco Laboratories, Detroit, MI) and was incubated at 37 °C overnight. After incubation, serial dilutions were performed to obtain approximately 10^2–10^4 cells of bacteria to be used for inoculation of water samples.

All inoculated water samples were incubated for 24 h at 37 °C without shaking, in sterile glass bottle, hermetically closed with an air volume of at least 1/10 of the volume of the culture. Just after the inoculum (t_0) and at the end of the 24 h (t_{24}) incubation, each sample (or aliquots) was filtered on nitrocellulose membrane (pore size 0.45 μm) (Whatman-GE Healthcare, USA) and transferred aseptically on agar plate, containing TSA (Tryptone soya agar, Oxoid), for *E. coli*, SB (Slanetz and Bartley, Oxoid), for *E. faecalis* and BPA (Baird–Parker agar, Oxoid) for *S. aureus*. In order to not have overcrowded plates, different dilutions/quantities of samples were filtered. Also not inoculated waters were analyzed in order to confirm the absence of external bacterial loads. The agar plates were incubated for 18 h at 37 ± 1 °C. The resulting colonies were counted, obtaining a value of colony forming units (cfu).

Microbiological inocula were performed in triplicate, and for each independent sample a set of three analyses was performed.

3. Results

3.1. Bactericidal activity of thermal spring waters

On the basis of chemical compounds present in the samples, the four thermal spring waters were classified as sulfate bicarbonate containing alkaline hearth metals (SBA), sulfurous containing alkaline hearth metals, sodium chloride, bromide and iodide (SSC), water with a higher content of arsenic and iron (AI), and sulfurous water with high levels of hydrogen sulfide (SW). The fixed residue at 180 °C was ranging between 2.5 and 3.3 g/L, and pH at 18 °C between 6.4 and 7.4. The highest concentration levels of H_2S were found in SW and SSC water, 29 mg/L and 18 mg/L, respectively, while the SBA and AI waters presented lower levels of H2S, ranging between <0.2 and 2 mg/L. The concentration of iodide and bromide ions was very similar in all 4 waters, whereas the content of sodium was variable spanning from 30 mg/L (SBA) to 600 mg/L (SSC). Out of the four thermal spring waters, only SW was hypothermal (>20–30 °C according to [23]) while the others were thermal (>30–40 °C) or hyperthermal (>40 °C) (Table 1).

Table 1
Chemical and physical characteristics respectively of the thermal waters utilized in the present work. SSC: sulfurous water containing sodium chloride, bromine and iodine. SBA: sulfate bicarbonate water containing alkali hearth metals. AI: water containing arsenic and iron. SW: sulfurous ipothermal water.

	Selected water samples			
	SSC	SBA	AI	SW
Source temperature (°C)	67	54	41	23.9
pH at 18 °C	6.8	6.5	7.4	6.45
Fixed residue at 180 °C (g/L)	2.5	3.3	2.9	2.9
H2S (mg/L)	18	2	<0.2	29
Iodide ions (mg/L)	2	<0.01	<0.01	l0.07
Bromide ions (mg/L)	5	<0.1	0.6	<0.1
Sodium (mg/L)	600	30	38	173