Full Length Research Paper

An outbreak of pneumonia in a thermal water spa contaminated with *Pseudomonas aeruginosa*: An epidemiological and environmental concern

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This article described an outbreak of bacterial pneumonia/bronchopneumonia in a group of visitors to a mineral water spa contaminated with *Pseudomonas aeruginosa* in summer 2009. The epidemiologic investigation was activated after the hospitalization for pneumonia of three people who had undergone therapy at the spa. Discharge with a diagnosis of pneumonia/bronchopneumonia in weeks 27 to 39 of 2009 of four hospitals near the spa were data-linked with the list of spa's visitors. Environmental samples of thermal water were performed and analysed. Investigations discovered a total of 39 cases of pneumonia among the spa visitors. Checks carried out in the hospitals near the spa revealed more hospitalizations for pneumonia in summer 2009 than the average for the previous five summers. There was a significant association between pneumonia and inhalation therapy (RR=7.33; p<0.0001) and aerosol therapy (RR=8.25; p<0.0001). *P. aeruginosa* was discovered in the water of the inhalation equipment. The spa micro-environment offers a risk of infection from pathogenic and/or opportunistic micro-organisms and therefore in-house testing, surveillance and prevention systems should be put in place for the well-being of clients.

Key words: Community acquired pneumonia, *Pseudomonas aeruginosa*, thermal water.

INTRODUCTION

The consumption of microbe infected water with the health risks entailed has always been a serious problem for public health. Mineral water springs, coming from deep underground, should be microbiologically pure and therefore not be a potential vehicle for spreading infection.

However, to safeguard consumers' health, spa must have a self-monitoring system of water quality and health authorities should carry out regular controls. Spa water quality must meet the requirements of the Ministry of Health Decree No. 542 of November 12 1992 which states the waters must not contain coliform, fecal streptococci, spores of clostridium sulfite reducers, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Italian Ministry of Health, 1992).

In the past, outbreaks of infection have been described with spa waters as the vehicle for the etiologic agent (Schaffler-Dullning et al., 1992; Hubert et al., 1991; Corona et al., 2008; Martinelli et al., 2001; Modi et al., 2008; Kawano et al., 2007).

This paper describes an outbreak of bacterial pneumonia/bronchopneumonia in a group of visitors to a hot-spring spa in Italy. The water of the spa was contaminated with *P. aeruginosa*.

In August 2009, a hospital in the Apulia Region informed the competent authorities of the hospitalization for bronchopneumonia of three patients who had recently undergone thermal inhalation therapy at a mineral water spa. This event immediately triggered an epidemiological investigation with the necessary environmental checks.

The spa offers treatments on behalf of the Health Service including inhalation therapy, aerosol therapy, nebulisation, nasal irrigation, endotympanic insufflation,

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mud bathing therapies, water therapy, mud therapy and thermal walkways, while private treatments such as massage, hydrokinesitherapy, physiotherapy and beauty treatments are also available. The spa waters are hypothermal and rich in mineral salts such as sulfates, magnesite and fluorides, and are slightly bromated.

These waters have been officially recognized by the Ministry of Health as having therapeutic properties for the treatment of respiratory, muscoloscheletric, venolymphatic, gastroenteric and gynaecological disorders.

In 2009, the spa was open from 4th May to 24th October and by 14th September had supplied 213,330 treatments to 18,282 clients.

MATERIALS AND METHODS

Benchmark analysis

To identify any excess of hospitalization for pneumonia and bronchopneumonia in the 2009 summer season, the Apulia Region hospital discharge database was utilized to reconstruct the historic series of admissions for the years 2004 to 2008, selecting records with the following criteria:

1. Week of hospital admission: from 27 to 39,

2. Type of admission: resident in the Apulia Region or not,

3. Discharge diagnosis: ICD-9-CM codes 480 (Viral pneumonia), 481 (Pneumococcal pneumonia), 485 (Bronchopneumonia organism unspecified), 486 (Pneumonia organism unspecified),

4. Discharge hospital (four hospitals near to the spa were identified as potentially those which would be used in case of need by spa clients).

Assessment of exposure and spa therapies

To assess the exposure we designed a cohort study of the persons treated in the spa during the specified weeks and for whom a diagnosis of pneumonia/bronchopneumonia was made. People who had inhalation or aerosol were considered as a case. People who had undergone mud therapy or other treatments were also considered as controls.

The Regional Health Authority asked the four hospitals to supply a list of those patients admitted to internal medicine wards (Pneumology, Internal medicine, Geriatrics) with a diagnosis of pneumonia/bronchopneumonia in weeks 27 to 39 of 2009. The list provided for each patient: name, surname, date of birth, personal code, date of hospital admission, admission diagnosis, date of discharge, discharge diagnosis.

After loading the admissions information for 2009 into a database, it was linked to the treatments database supplied by the spa, and analysis was carried out to identify the subjects who had developed pneumonia/bronchopneumonia within fifteen days after the end of the spa treatment.

The database contained: name, surname, date of birth, personal code, treatment received in the spa, date of the treatment of each person treated.

Statistical analysis

Chi-square for linear trend was used to evaluate the increased of

hospital admission in 2009. To analyse the associations between exposure and pneumonia/bronchopneumonia, the independent samples t-test was used in the analysis of the difference between continuous variables; the categorical variables were classified in double entry contingency tables and the values of the chi-square test, relative risk and the relative confidence intervals at 95% were calculated. A p-value <0.05 was considered significant for all tests. Data was analysed using the statistical software Epi-Info 6.00 (public domain software - CDC Atlanta, Georgia; WHO Geneva, Switzerland).

Environmental sampling

The health authorities, following the notification, inspected the spa on 27th August 2009, performing hygiene and health checks and taking 9 water samples of 1 L from each of the following: a nasal irrigation booth, an aerosol booth, a micro-nebuliser tampon, the spa swimming pool, a whirlpool bath, a shower in the bathing therapy section, a mud therapy booth and two hand basins, one in physiotherapy and one in a doctor's surgery. A further 10 samples were taken from the showers and taps in some rooms in the hotel in the complex. A second randomised sampling was carried out on 7th September in 26 points, 15 in the spa and 11 in the hotel. In the spa, the sampling points were 1 inhalation booth, 1 nasal irrigation booth, 1 in the swimming pool, 1 in a swimming pool shower, 1 in the walkway in the spa pool, 3 whirlpool baths, 3 showers in the changing rooms, 2 from the thermal water source, 1 from the Olympic swimming pool and 1 from the Olympic pool shower. In the hotel, sample points were 1 from the hotel pool, 1 from the hotel pool showers, 5 from room showers and 4 from room taps.

Further sampling was performed in 17 aerosol booths on 15th September and in 36 aerosol booths on 17th September 2009.

Testing was performed on all samples for *Legionella pneumophila* and *P. aeruginosa* according to the guidelines for testing drinking water for *Pseudomonas* (Standing committee of Analyst, 2002) and *Legionella* (Permanent Conference for Relations between State, Regions and Autonomous Provinces of Trento and Bolzano, 2005). Initial testing of the water samples taken on 27th August was performed by the Regional Agency for Environmental Protection, while all other testing was done in the laboratory of the Hygiene Department of the University Hospital, Bari.

Interventions undertaken in order to contain potential transmission

In the period between the samplings, the spa management had sanitised the spa waters with hydrogen peroxide. On 8th and 11th September the spa water pipes were sanitised with high strength sodium hypochlorite and on 10th and 11th September the airconditioning system was sanitised.

Microbiological testing on patients

In the date 27th August 2009 the health authorities asked the four local hospitals to conduct microbiological exams on all the patients admitted for pneumonia/bronchopneumonia who had had a recent treatment at the spa. Samples were sputum, nasopharyngeal swab and, if possible, liquid from bronco-alveolar lavage. These were sent to the laboratory of the Hygiene Department of the University Hospital, Bari and subjected to microbiological testing, particularly for *L. pneumophila* (Permanent Conference for Relations between State, Regions and Autonomous Provinces of Trento and Bolzano, 2005), *P. aeruginosa* (Gilligan, 2001), fungi and the A/H1N1 virus (Panning et al., 2010).

Table 1. Admission for pneumonia/bronchopneumonia inthe weeks 27/39 in the 4 hospitals near the spa. Years2004 to 2008.

Year	Admitted
2004	117
2005	129
2006	142
2007	163
2008	128
Average 2004/2008	135.8

RESULTS

Benchmark analysis

The average number of admissions for pneumonia/ bronchopneumonia in the weeks 27/39 in the four hospitals near the spa in the period 2004/2008 was 135.8/season (Table 1) with an average age of discharged patients of 61.2 years (SD=25,7; range 0-99).

Analysis of patients admitted in 2009

In the four hospitals, for the weeks 27 to 39 of 2009, there were 186 admissions, more than any of the previous five years, with an average age of 65.9 years (SD=22,6; range 0-99) and a median age of 62 years. (Graph 1 shows the distribution of admissions by week). From week 31 on, there were more admissions than the previous five-year weekly averages for the four hospitals (Graph 2).

Chi-square for linear trend test did not show an increase of number of hospital admission in the weeks of 2009 analysed (chi-square=3, 14; p=0,07).

There were 39 patients with previous recent exposure to inhalation or aerosol therapy, average age 61.6 years (SD=18.2; range=10-86) and median age 68 years. Symptoms arose during therapy in 23 patients, 1 to 5 days after therapy in 14, after 8 days in 1 and after 11 days in the last. Among these, 32 had had inhalation therapy and 32 had had aerosol therapy. There were a further 6 pneumonia/bronchopneumonia patients who had had mud bathing therapy at the spa. The trend in pneumonia/bronchopneumonia cases during the weeks under investigation in patients exposed and not exposed to inhalation and aerosol therapy at the spa are shown in Graph 3.

During the weeks 27 to 39 of 2009, 15,829 people had therapy at the spa, giving an incidence rate for pneumonia/bronchopneumonia of 2.5/1000 customers. The incidence rate for pneumonia/bronchopneumonia was: 4.3/1000 for patients who had had inhalation therapy and 0.6/1000 for those who had not had inhalation therapy; and 4.6/1000 for patients who had had aerosol therapy and 0.6/1000 for those who had not had aerosol therapy. For mud therapy, the incident rate was 1.8/1000 for exposed patients and 2.9/1000 for not exposed. For other therapies the rates were 0.5/1000 exposed and 4.7/1000 not exposed. The relative risk of pneumonia/bronchopneumonia from the various therapies is shown in Table 2.

Environmental sampling

Testing completed on 4th September by the Regional Agency for Environmental Protection for *Legionella spp* was negative in the samples collected on 27th August, but three samples, from a whirlpool bath, a shower and a washbasin, had developed unidentified colonies of bacteria suggesting other microbiological pollution. Further testing by the laboratory of the Hygiene Department of the University Hospital, Bari confirmed the lack of *Legionella spp* and found *P. aeruginosa* of serogroup P11 and *Candida albicans*.

The presence of *P. aeruginosa* in the spa water was confirmed by microbiological testing on the samples taken on 7th September (Table 3), 8 of the 15 being positive with concentrations ranging from 25 to >500 cfu/250 ml. *P. aeruginosa* was also found in 2 of the 11 samples from the hotel showers and taps.

The results of testing on the samples taken on 15^{th} September, after sanitizing, was positive for *P. aeruginosa* in all 17 aerosol booths, 15 of them with bacterial loads greater than 500 cfu/250ml, while the other two resulted 100 cfu/250 ml and 120 cfu/250 ml. The serogroups were P1, P6, P8, P11, and three non-typable strains.

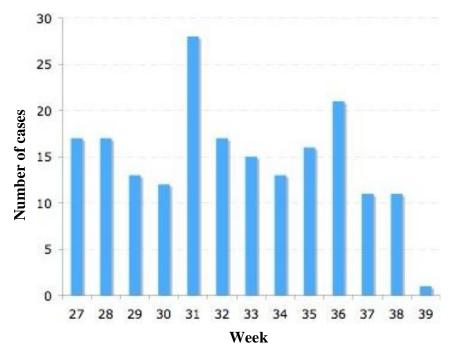
The sampling of 17th September, performed in 36 aerosol, found *P. aeruginosa* in 7 booths with loads of 1, 150, 15, 3, 12, 40 and 1 cfu/250ml. Serotyping identified groups P6, P9, P11 and 2 non-typable strains.

Microbiological testing on patients

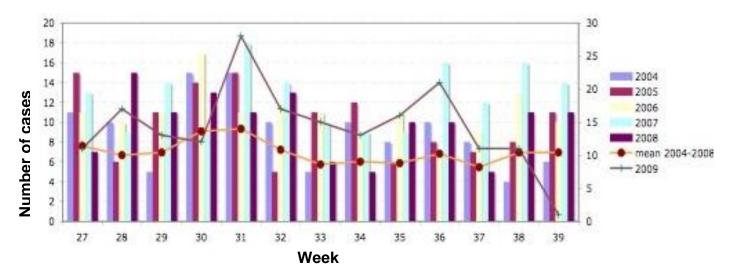
In September, samples of sputum, nasopharyngeal swab and liquid from bronco-alveolar lavage were taken from only 9 patients, who, at sampling, were following a combined antibiotic therapy. All patient samples were negative for virus 2009 A/H1N1 and *L. pneumophila* and all samples were also negative for *P. aeruginosa. C. albicans* was isolated from the samples from 2 patients.

DISCUSSION

The results of the investigation may suggest a possible exposure to contamination by *P. aeruginosa* in the customers of the spa who had inhalation or aerosol therapies and, following this exposure, developed acute respiratory infections in the pulmonary parenchyma



Graph 1. Number of cases pneumonia/bronchopneumonia hospitalised in our hospitals near the spa, by week of admission. Year 2009, weeks 27/39.

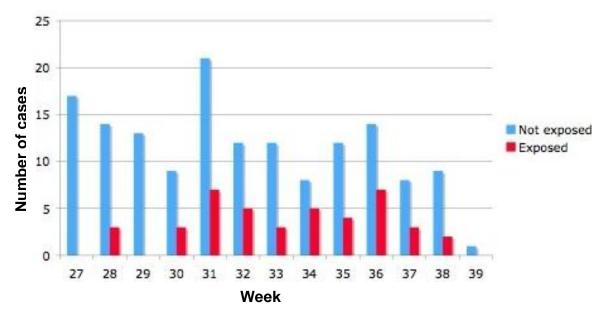


Graph 2. Number of cases pneumonia/bronchopneumonia hospitalised near the spa, by week of admission. Years 2004-2008 and 2009, weeks 27/39.

and/or the bronchial tree, even if it was not found in the small number of biological samples taken from affected patients. The presence of *C. albicans* in biological samples from two patients, does not seem to indicate exposure not only because of its infrequency in the samples taken and because of the known biology of fungi which is invasive only in seriously immunocompromised patients, but also because of the frequency with which it

is found as part of the commensal flora in the upper respiratory tracts of healthy people.

However certain considerations must be made on the limits of our observations. Even though the waters of mineral water spas can furnish ideal conditions for the proliferation of opportunist pathogens like *P. aeruginosa* or *L. pneumophila*, the waters are generally considered to be microbiologically pure at source and so spas do not



Graph 3. Number of cases pneumonia/bronchopneumonia hospitalised near the spa, by exposure to inhalation and aerosol therapy. Year 2009, weeks 27/39.

Table 2. Relative risk between exposure to spa therapy and onset of pneumonia/bronchopneumonia.

Spa therapy	RR	95% CI	Chi-square	Р
Inhalation	7.33	2.86-18.81	23.7	<0.0001
Aerosol	8.25	3.08-24.77	27.6	<0.0001
Mud	0.61	0.23-1.51	1.29	0.25
Other	0.12	0.05-0.30	28.72	<0.0001

carry out routine disinfection. However, it cannot be discounted that anthropization and wear and tear of equipment can over time allow the establishment and growth of microbial colonies. For this reason the design of the water systems is of great importance to prevent the formation of bio-film. Water flows must be adequate, the use of certain materials and components must be avoided and the natural characteristics of the waters must be maintained (Italian National Institute of Public Health, 2000).

A review of the literature on outbreaks of infections linked to spas discovered various cases of legionellosis. In fact, in Italy, the spa of San Pellegrino Terme in Bergamo was closed as a precaution in 2002 (Ferrara et al., 2009). However, there was very little information on acute respiratory infections. In 1987, in France, there was an outbreak of infection caused by a previously undescribed Gram-negative bacterium which affected people attending a hot spring spa. 35 cases of pneumonia and two cases of meningitis occurred. An epidemiological comparison of the 26 hospitalized cases with 52 matched controls suggested that spa treatments were significant risk factors for acquiring the infection and later the same bacterium was isolated from the hot spring water (Hubert et al., 1991). Skin disorders have also been recorded, caused by the presence of *P. aeruginosa* in regularly used whirlpool baths (McCausland et al., 1975; Washburn et al., 1976; Feder et al., 1983) which have favourable factors like abundant nutriment such as cosmetics, skin fragments, bacteria, fungi and other elements. However the literature did not reveal evidence of outbreaks of pneumonia/bronchopneumonia involving *P. aeruginosa* in spas.

That *P. aeruginosa* is a plausible biological cause of contamination is well supported by evidence, at least theoretically. It is a ubiquitous micro-organism found in surface water, waste water and the sea, in soils and vegetation, and everywhere there is humidity. It has also been found in chlorinated water, it has a high resistance to mechanical cleaning and pressure, and to disinfectants and antibiotics. It multiplies rapidly in high water temperatures, like those in hot water spas (Grobe et al., 2001).

P. aeruginosa is a bacteria, which is frequently implicated in illnesses and epidemics associated with the use of bathing establishments, and the second most commonly found in hospitals (McCausland et al., 1975; Edwards et al., 2008). An investigation carried out in Italy in 2005 found *P. aeruginosa* together with *L. pneumpophila* and Mycobacteria in samples taken from an aerosol system in a spa in the region of Emilia Romagna. Treatments with sodium hypochlorite and peracetic acid worked well against the microbial infection and eliminated *Pseudomonas spp* in the water storage tank, but were not effective in the nasal irrigators and

Sample point	Presence of pseudomonas (ufc/250 ml)	Serogroup
Spa		
Nasal irrigation booth	>500	P4
Whirlpool bath***	25	P1
Changing room shower***	>500	n.i.
Whirlpool bath***	50	P11
Changing room shower***	>500	P1
Whirlpool bath***	30	n.i.
Changing room shower***	300	P6
Olympic pool shower	10	P1
Hotel		
Shower by swimming pool	12	P6 + P11
Room taps n.***	5	P11

Table 3. Results of research for Pseudomonas aeruginosa in water samples taken 7th September 2009.

All other samples were negative; n.i.: not identifiable.

nebulisers, demonstrating that the disinfection did not reach all points in the distribution circuit of the spa waters. To be able to disinfect the water distribution lines at delivery point of *Legionella spp* and *Pseudomonas* spp, a radical restructuring of the water delivery system was necessary, using new technology to allow periodic thermal shock treatment (Leoni et al., 2005).

Furthermore, mineral water spas are often attended by the elderly and the immune-depressed who are at higher risk of infection. In the 2009 season, the average age of the customers of the spa was 62 years, while it was 68 years for the patients who developed pneumonia/ bronchopneumonia and had undergone spa therapy. Immunosenescence can without doubt lower resistance to infection and reduce the ability of the patient to combat the progression of diseases correlated to *P. aeruginosa*, which is principally known as an opportunistic pathogen.

Finally, it is worth noting that the role of *P. aeruginosa* has been documented in the etiology of pneumonia and bronchopneumonia within the community and within hospitals and that the spa micro-environment is not at all dissimilar to that of the hospital.

The discovery of *P. aeruginosa* in the waters of the spa and the number of pneumonia cases in exposed patients in the weeks prior to the beginning of the investigation lead to a strong suspicion that the spa's system of checks and controls was not functioning properly. That the quality of the water was not within the laid down norms should have been discovered and the correct corrective procedures taken to safeguard public health.

From the methodological point of view, the study is limited by the impossibility of systematically examining the biological samples of all the patients before treatment was commenced. In fact less than 25% of the patients had samples taken and that only after the start of empiric broad-spectrum antibiotic treatment which can render diagnostic tests ineffective. This limitation was caused by the delay in identifying a possible correlation between exposure to the spa therapies and disease onset, the source being identified only two months after the initial outbreak thanks to the work of one of the few permanent Hospital Infection Control Panels within the Puglia Region of Italy. It should also be pointed out that the empiric approach to antibiotic therapy is more and more widespread especially for serious and less serious infections, which, in Puglia, is also related to the physical and organizational problems of the microbiology laboratories.

This limitation of no finding of *P. aeruginosa* in patient samples does not allow us to confirm that the spa and or its therapies was the source of the infection but at the same time it does not exclude this possibility.

Another aspect which must be considered is that the patients hospitalized for pneumonia/bronchopneumonia may represent only a small fraction of the respiratory pathologies related to spa therapies, only the most serious cases being admitted to hospital. However the large number of customers did not allow any assessment of potentially exposed clients to be carried out.

Some words must be spent on the usefulness of epidemiologic investigation in a context which, other than being a public health problem, is characterized by the determining of legal responsibility. In Italy, epidemiologic investigations have been carried out for legal purposes, usually because of local pressure groups, to show any links between environmental exposure to pollution and neoplasias. The most notable were probably that of the Public Health Agency of the Lazio Region in 1997 to 2001 regarding electromagnetic pollution from Radio Vatican and the high rate of leukemia mortality in Lazio and that of the high rate of hepatic angiosarcoma deaths among employees of Enichem in Porto Marghera in the Veneto Region (Marini, 2002; Pirastu et al., 2003). In Italy, in law and its interpretation, objections have been made to the applicability of the results of epidemiological studies to criminal proceedings in that epidemiology and its methods are based on statistical analysis which does not allow absolute certainty in cause and effect while penal responsibility requires proof beyond any reasonable doubt. Conversely, the appearance of pathologies known to be linked to exposure, in absence of actus reus, is a liability in civil law, where the value judgement is at the level "more likely than not" (IVD Spinea Venerzia, 2001).

However, the cluster that we have described in this work is an infective disease and not a chronicdegenerative pathology like the tumours mentioned in the surveys above. Infective diseases are conditioned by deterministic factors different from those for chronic diseases. Exposure to the agent is not a risk factor but a necessary cause, though not sufficient in itself, to trigger the disease, which leads to the suggestion that new aspects of the law and its interpretation must be found.

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