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Article

Balneotherapy is a potential risk factor for *Pseudomonas aeruginosa* colonization

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The practice of immersion in burn patient has been abandoned in many parts of the world but in Brazil it is still common. The aim of this study was to ascertain if balneotherapy is a risk factor for *Pseudomonas aeruginosa* colonization in thermally injured patients. Eighteen patients from a Burn Center were studied for 14 weeks for *Pseudomonas aeruginosa*. Samples were collected by swabbing the exudate of wounds, before and after giving bath to the patients and from balneotherapy table. Pulsed-field gel electrophoresis was used to determine bacterial genetic relatedness. Thirty-seven *P. aeruginosa* isolates were detected from 292 swabs collected from patients' burn surface area and from the balneotherapy table. Profile analysis of *P. aeruginosa* DNA fragmentation showed 10 clones among the 37 strains analyzed. Type A is the most prevalent clone, with 23 strains distributed into eight subtypes. These were present in the swabs collected, before and after the patients' bath, from the surface of the bath table, suggesting that there was cross-contamination between the patients in different ways. This work demonstrates that balneotherapy is a risk factor in the Burn Center studied, because the same clone was found among *P. aeruginosa* isolates collected at various points and times.

Uniterms: *Pseudomonas aeruginosa*/colonization. Burn patients. Balneotherapy. PFGE.

A prática de balneotarapia em paciente queimado foi abandonada em muitas partes do mundo, mas no Brasil ainda é comum. O objetivo deste estudo foi verificar se a balneoterapia é um fator de risco para a colonização por *Pseudomonas aeruginosa* em pacientes queimados. Dezoito pacientes internados em um Centro de Queimadura (CQ) foram acompanhados por 14 semanas. Amostras foram coletadas do exsudato de feridas, antes e depois do banho dos pacientes e também da mesa onde a balneoterapia foi realizada. A relação genética entre as cepas de *P. aeruginosa* foi determinada pela electroforese em gel de campo pulsado. Trinta e sete cepas foram detectadas a partir de 292 swabs coletados de área de superfície das feridas dos pacientes e da mesa de balneoterapia. Análise de fragmentação do DNA das 37 *P. aeruginosa* mostrou a existência de 10 clones. O tipo A foi o clone mais prevalente, com 23 cepas distribuídas em oito subtipos. Estas estavam presentes nas lesões dos pacientes antes e após o banho e na mesa onde o banho foi realizado, sugerindo contaminação cruzada inter e intra-pacientes e pacientes e mesa de banho. Este trabalho mostra que a balneoterapia é um fator de risco para colonização por *P. aeruginosa*, no CQ estudado, pois um mesmo clone da bactéria foi encontrado nos isolados coletados em vários pontos e épocas diferentes.

Unitermos: *Pseudomonas aeruginosa*/colonização. Pacientes queimados/tratamento. Balneoterapia. Eletroforese em campo pulsado.

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INTRODUCTION

Infection is a major cause of morbidity and mortality of burn patients (Elmanama *et al*., 2013; Mahar *et al*., 2010). These patients are highly susceptible to infections, because by then they have lost the integrity of the skin, the first barrier of protection against infectious agents. Besides, they may require hospitalization for long periods, which increases the risk of infection (Church *et al*., 2006).

Balneotherapy or hydrotherapy in burn patient can be done in designated areas only that ensure control and prevention of infection. The burn wound is washed and scrubbed with water and degermed by a spray hose. During this procedure, topical agents, necrotic tissue and microorganisms are removed (Kowalske, 2011). However, the practice of immersion has been abandoned in many parts of the world. Notwithstanding, in some Burn Center (BC) in Brazil it is still used.

Some authors consider that colonization of the burned area does not necessarily represent a risk factor for infection (Tredget *et al*., 2004; Wang *et al*., 2010). But, when there is contamination of the living tissue underlying the injury, with clinical signs of inflammatory reaction and browning, one can infer that the burned surface area (BSA) is infected and may lead to a systemic infection (Ulkur *et al*., 2005).

In burn patients, particularly those with severe burns, *P. aeruginosa* can cause a great variety of systemic infections, such as urinary tract infection, respiratory system infection, dermatitis, soft tissue infection, bacteremia, bone and joint infection and gastrointestinal infection (Taneja *et al*., 2013; Yali *et al*., 2013). Burned patients present an immunosuppression condition; they consequently have higher susceptibility to infections by *P. aeruginosa,* with a high mortality rate. In addition, as prevention and control of *P. aeruginosa* infections continue to be serious nosocomial problems worldwide, treatment of *P. aeruginosa* infections is a serious medical challenge especially in burned patients (Taneja *et al*., 2013; Yali *et al*., 2013; Fallah *et al*., 2013).

Therefore, prophylaxis with antibiotics is used for preventing or reducing the risk of infections in thermally injured patients (Barajas-Nava *et al*., 2013). Some antibiotics are used locally on the skin (topical treatment), while others are taken systemically.

Silver sulfadiazine (SSD) cream is one of the topical agents that is most commonly used as an adjuvant in the prevention and treatment of wound infections in patients with severe burns. In addition, it has broad antimicrobial spectrum on microorganisms found on BSA, with prolonged action. It is believed that bacteriostatic and bactericidal effect of SSD increases when it is associated with 0.4% cerium nitrate (De Gracia, 2001; Eski *et al*., 2011).

Molecular methods are often used in epidemiologic studies to monitor the spread of a particular strain in the same patient or environment or between different patients and environments. From such monitoring, it is possible to decipher the transmission routes of the bacteria (Nonaka *et al.,* 2010). Pulse-field gel electrophoresis (PFGE) is a gold standard genotypic technique for *P. aeruginosa* (Hu *et al*., 2013; Nonaka *et al*., 2010).

The aims of this study were to assess the role of the balneotherapy as a risk factor for burned patient as well to investigate the colonization by *P. aeruginosa* in the same patients to evaluate the efficacy of balneotherapy in its elimination.

STUDY SETTING

The BC studied belongs to a hospital in Rio de Janeiro city, Brazil. This center has 12 beds, of which eight are for adult patients and four for children. Generally, the patient's hospital stay is prolonged, ranging from 30 to 180 days.

At this center, we conducted an observational study for 14 weeks (September to December 2012) with prospective analysis. The inclusion criteria for the subjects of study were adult patients (+18 years old) admitted to the BC, whose bath samples can be collected at least on two different days.

METHODS

Management of burn patients

At this burn unit, all patients, after disinfecting the burned area with a degerming application, are submitted to a daily balneotherapy session. However, in this paper only one table was studied and approximately 5 to 6 patients received sequential treatments in the immersion table. SSD is applied for topical treatment of both superficial and deep burns. The Human Research Ethics Committee from HUAP at Universidade Federal Fluminense approved this study by number 68538.

Bacterial isolates and identification

Samples from burn surface area (BSA) were collected every Monday from each patient by swabbing the exudate of wounds immediately after removing the SSD dressing, but before giving bath to the patient. After giving bath and before SSD dressing, samples were collected

again from the previous sampling points, regardless of the clinical condition of the patients. Prior to sampling, the wound was rinsed with sterile saline 0.9% to eliminate any trace of SSD, which is applied daily for topical treatment.

Swabs also were collected from the table where balneotherapy was realized, before each bath and after the last bath. Between every two baths, a cleaning procedure was performed by the cleaning staff. Water analysis was performed once a month.

All swabs were inoculated into 2 mL of salt solution (0.9%), vortex and 1 mL was introduced into Tryptone Soya Broth (TSB; HIMEDIA, India) in double concentration. The samples were incubated at 35 °C (± 2) for 24 to 48 hours. The tubes that showed turbid medium were screwed onto cetrimide agar (HIMEDIA, India) and incubated again at the same conditions mentioned above. The colonies that could grow in cetrimide agar were submitted to testing for *P. aeruginosa,* using the standard biochemical tests. All the isolates were kept on crioprotector medium at -20 °C. To confirm detection of *P. aeruginosa*, polimerase chain reaction (PCR) was carried out according to Spilker *et al.* (2004).

Determination of minimal inhibitory concentration (MIC)

The MIC values were determined by agar microdilution method, using *Pseudomonas aeruginosa* ATCC 27853 as microorganism control. Muller Hinton agar (MHA) medium (DIFCO LABORATORIES, USA) was prepared with serial diluted concentrations of SSD (PHARMA NOSTRA, India) incorporated into agar. Steers replicator was used to transfer the inoculum to the plate.

Characterization of the isolates by PFGE

Clonal relatedness of the isolates was evaluated by PFGE as described previously (Gautom *et al.*, 1997), using the *SpeI* restriction enzyme instead of *XbaI* performed in a contour-clamped homogeneous-electric-field DRIII apparatus (Bio-Rad Laboratories, Hercules, CA., USA). The clonality was determined by visual analysis using Tenover criterion (Tenover *et al*., 1995).

Statistical analysis

Descriptive statistics was used for exploratory analysis of the variables involved in balneotherapy, and McNemar, a non-parametric test, was employed for risk factors analysis.

RESULTS

The results concerning the balneotherapy of 18 patients, for 14 weeks, were analyzed. Seventy baths, with a mean of 5 (± 0.6) patients per day bath, were monitored. The average time spent by a patient in each balneotherapy session was 29 minutes.

From the 292 swabs studied, 37 strains were identified as *P. aeruginosa*; of these, 28 strains were collected from BSA and nine from the balneotherapy table surface (BTS). Nine patients (50%) showed no bacterial colonization at any time of the study. Ages of the patients varied from 19 to 77 years (mean age=41, sd=18), but no extreme age patient was present in the group where *P. aeruginosa* was identified.

The gender distribution of the 18 patients tested was equitable (50% males and 50% females). Despite the isolates being few, it is remarkable that six out of nine patients, who tested positive for *P. aeruginosa*, were females. No relationship was observed between the percentages of BSA and colonization by *P. aeruginosa,* because patients with both 8.5% and 80% BSA were colonized by this microorganism. In addition, of the 28 *P. aeruginosa* isolates found in BSA, 20 were detected in female patients (Table I).

No *P. aeruginosa* isolates were detected in the water used for bathing the patients or in the material used to clean the table. Temporality assessment of *P. aeruginosa* colonization reveals an increase in bacteria during the last weeks of the study.

The first *P. aeruginosa* detected in this study was isolated from BSA and obtained on the fourth bath-day (day which the swab was collected) from the third patient before being submitted to the procedure.

The analysis of the total DNA fragmentation profile by PFGE demonstrated the existence of 10 clones among 37 strains of *P. aeruginosa* strains analyzed. Clone A was the most prevalent with 23 strains (62%), distributed into 08 subtypes (A1-A8). The majority of the patients were colonized by clone A in different moments of the study, except the patient 8 and 9 (P8 and P9; Figure 1). This clone was also found on the table surface after the balneotherapy sessions 13 and 14 (Table I).

Other PFGE patterns were also found and designated as C, D, E, F, G, H, I and J. Profiles classified as C, D, G, H and I were detected only in the BSA of patients, and H and J on the table (Table I). Three patients (P10, P11 and P17) had *P. aeruginosa* identified several times during the study (Figure 1). PFGE pattern could not be determined by *Spe*I in two samples (Table I).

The temporal analysis suggested the ineffectiveness

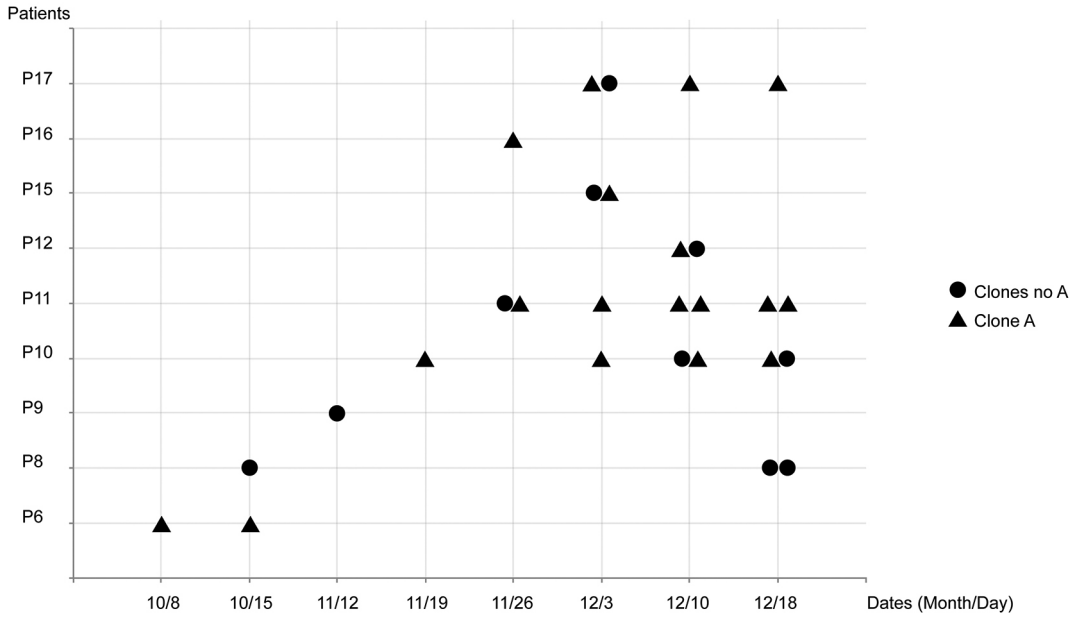

FIGURE 1 - Temporal analysis of the *Pseudomonas aeruginosa* found in the patient´s swabs.

of the balneotherapy (Figure 1), although the number of samples did not allow the identification of a significant statistical difference neither for the bath (odds ratio=1.200; 0.305 < CI 95% < 4.971; p = 1.0000) nor for the table (odds ratio= 0.333; 0.006< CI 95% < 4.151; p = 0.6171) as a risk factor.

In addition, this analysis could also show as *P. aeruginosa* was spread to different patients. The subtype A2 was found in different patients (P6, P10, P11 and P16) and in different moments. Similarly, the subtype A3 was found after and before the P11 bath. These examples suggest the hydrotherapy was not effective (Table I).

Because silver sulfadiazine was extensively used in the treatment of burned patients, MIC for this antimicrobial was determined for 37 *P. aeruginosa* isolated in this study. The results demonstrated that MIC values ranged from 32 mg/L to 128 mg/L;

32 mg/L in eight isolates, 64 mg/L in four and 128 mg/L in most isolates (Table I).

DISCUSSION

P. aeruginosa, an important source of nosocomial infections, can be acquired in a community or hospital setting. It is the most common opportunistic pathogen to infect hospitalized burn patients (Fallah *et al*., 2013; Ackerman *et al*., 2013). According to Ackerman *et al* (2013), patients with severe burns have immunosuppression, and consequently, increased susceptibility to nosocomial infections by *P. aeruginosa,* resulting in high mortality rates, mainly because of their high intrinsic resistance to many antibiotics.

In this study, 37 *P. aeruginosa* isolates were detected from the BSA of 9 out of 18 patients. In addition, this microorganism was identified on the table surface where balneotherapy occurred.

Although in this study we observed *P. aeruginosa* colonizing more female patients, we could not investigate the role of gender as a risk factor, owing to the small number of patients with *Pseudomonas* colonization. Moreover, some authors find no significant statistical difference between the results of patient cultures and their genders (Al Laham *et al*., 2013).

Molecular typing methods using PFGE are important tools for epidemiological studies (Ballarini *et al*., 2012; Mudau *et al*., 2013). The similarity between strains allows determination of the source of an outbreak, and facilitates implementation of effective control measures (Mudau *et al*., 2013).

In this work, we detected one clone (named A) as a major *P. aeruginosa* clone spread during all period of the study (Figure I). This clone was found on the surface of the table among multiple procedures and on BSA patient suggesting transmission of the isolate from table to patient (Table I).

Persistence of clones of *P. aeruginosa* in the hospital environment has been discussed by several authors (Tredget *et al*., 2004; Wolska *et al*., 2012; Johansson *el al*., 2014). Analysis of table surface demonstrates possible cross-transmission of the microorganism during

TABLE I - Characterization of the 37 colonizing *Pseudomonas aeruginosa*

Isolate	Patient report			Collection date	Collection site	Moment of collection	[d]PFGE	[e]MIC silver sulfadiazine
	Gender	[a]BSA	Age					
1	F	30%	23	10/08/2012	[b]P6	Before bath	A1	128
2	M	48%	48	10/15/2012	P8	After bath	D	128
3				10/15/2012	P6	Before bath	A2	128
4	-	-	-	10/22/2012	[c]BTS	Final cleanup	E	128
5	M	80%	25	11/12/2012	P9	After bath	NT	128
6	F	30%	34	11/19/2012	P10	After bath	A2	128
7	F	44,5%	24	11/26/2012	P11	Before bath	F	32
8				11/26/2012	P11	After bath	A2	64
9	F	8,5%	27	11/26/2012	P16	After bath	A2	128
10				12/03/2012	P10	After bath	A5	128
11				12/03/2012	P11	Before bath	A6	128
12	M	18%	54	12/03/2012	P17	Before bath	A6	128
13				12/03/2012	P17	After bath	G	32
14	F	18,5%	59	12/03/2012	P15	Before bath	H	128
15				12/03/2012	P15	After bath	A1	128
23	-	-	-	12/10/2012	BTS	First cleanup before the patients' baths	A1	32
16				12/10/2012	P10	Before bath	I	128
17				12/10/2012	P10	After bath	A4	64
24	-	-	-	12/10/2012	BTS	After the first bath	A1	128
18	F	14%	34	12/10/2012	P12	Before bath	A4	128
19				12/10/2012	P12	After bath	H	64
25	-	-	-	12/10/2012	BTS	After the second bath	-	128
20				12/10/2012	P17	Before bath	A7	128
26	-	-	-	12/10/2012	BTS	After the third bath	A1	32
21				12/10/2012	P11	Before bath	A1	128
22				12/10/2012	P11	After bath	A8	128
27	-	-	-	12/10/2012	BTS	After the fourth bath	A2	128
28	-	-	-	12/10/2012	BTS	After the fifth bath	A1	128
31				12/18/2012	P8	Before bath	C	128
32				12/18/2012	P8	After bath	NT	64
33				12/18/2012	P17	After bath	A1	128
38	-	-	-	12/18/2012	BTS	After the third bath	J	32
34				12/18/2012	P11	Before bath	A3	128
35				12/18/2012	P11	After bath	A3	32
39	-	-	-	12/18/2012	BTS	After the fourth bath	F	32
36				12/18/2012	P10	Before bath	A5	128

[a]BSA: burn surface area. [b]P: patient identification. [c]BTS: isolates collected from the balneotherapy table surface. [d]PFGE: pulsed-field gel electrophoresis pattern. [e]MIC: minimal inhibitory concentration.

balneotherapy procedure (Table I). This microorganism is known for its ability to adhere to both biological cell membranes and inert surfaces, mediated through the pili and by the production of large amounts of exopolysaccharides (Pier *et al*., 1987). In addition, this microorganism has strong capability to develop multidrug resistance to many drugs (Ackerman *et al*., 2013; Shanthi *et al*., 2013). Additionally, Tredget *et al*. (2004) demonstrated that *P. aeruginosa* clinical isolates, belonging to the same clone, were present in both the patients and the sink trap. In this study, transmissions from patient to patient and patient to balneotherapy table were verified. It seems that some isolates have greater ability to persist and fit better in the environment at the expense of others (Elmanama *et al.,* 2013). In this study, although only a few isolates were detected, we can verify the predominant clone persisted for 14 weeks (Figure 1). Possibly, the clone is better adapted to the conditions of the study.

Analysis of the frequency of subtype A2 demonstrates the transmission of the isolate between two patients (Table I), besides confirming the isolate's ability to persist. A3 subtype isolate demonstrates the ineffectiveness of balneotherapy for decolonization, because the same isolate was found before and after balneotherapy of the same patient.

Regardless of clonality, evaluating contamination by *P. aeruginosa* as a risk factor might deserve attention. Although no statistically significant relationship could be observed among balneotherapy procedures, probably because of fewer patients and fewer events, the fact that six patients, who were not contaminated before bathing, became contaminated after bathing, suggests that bathing can be considered a clinically risk factor. In addition, eight patients who were found as contaminated before balneotherapy remained so even after bathing (Figure 1). This leads one to believe that neither the procedure nor the degermant allowed decontamination. This observation is reinforced by the genetic profile analysis of DNA bacteria showing similarity among isolates collected at points and different moments.

The risks associated with the ways the injury is taken care of, such as daily bath, can be related to the source of water (tap, hose or shower), which is frequently contaminated with microorganisms of the environment, such as bodies of other patients (Tredget *et al*., 2004). For this study, besides the bath table, the water used was also evaluated and no contamination by *P. aeruginosa* was observed.

Silver sulfadiazine serves better to prevent wounds colonization/infection and therefore is the best choice to reduce the risk of sepsis, a serious complication and a major threat to burn victims with large burns (Shanthi *et al*., 2014; Hajska *et al*., 2014). At the BC studied, SSD agent was used as a dressing reference standard therapy. It is important to note that in this study, 64% of the isolates tested showed MIC of 128 mg/L (Table I). This suggests decreased susceptibility of the bacteria in comparison to the findings of a study conducted in 1973, in New York, which reports that 100% of the strains of *P. aeruginosa* infection of skin had MIC of 50 mg/L and 60% of the isolates tested showed MIC of 6.25 mg/L (Carr *et al*., 1973). We suggest that the wide use of this topical agent could select more resistant strains to this antimicrobial.

CONCLUSION

The temporal analysis of balneotherapy in decolonization of SCQ by strains of *P. aeruginosa* shows that this procedure is not effective for decontamination of patients and table. On the surface of the table where the baths were realized, clones of the same subtype of *P. aeruginosa* were found, suggesting that the table was not being adequately disinfected. In addition, clones of the same subtype of *P. aeruginosa* were found, before and after bathing, in different burn areas of the same patient. This suggests cross-contamination between patients.

The MIC values obtained in this study for silver sulfadiazine are rather higher in comparison to those reported in different decades, suggesting that other techniques must be used for topical treatment of burned patients.

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There are no conflicts of interest to report for this study.

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