**TWEED LABORATORY CENTRE**
**ABN 90 178 732 496**
46 Enterprise Avenue, Tweed Heads South, NSW 2486
Ph: 07)5569 3100 Fax: 07) 5524 2676

# *Pseudomonas aeruginosa* Disinfection In Spa Pools

## Laboratory Experiments conducted with Enviroswim System for WaterTech Services International Pty Ltd.

**Paul Wright PhD.**

## *Introduction*

Copper and silver ionisation of water has become an emerging method for disinfection in both swimming and spa pools. WaterTech Services International Pty Ltd has developed a new disinfection system incorporating copper and silver ionisation which they have operating in both public and private swimming/spa pools in Queensland.

Our laboratory was approached to do some efficacy testing to determine the system's ability to disinfect spa pool water. Guidelines for measuring the efficacy of a disinfection system have been drawn up by the NSW Health Department ("Treated Water Public Swimming Pools and Spa Pools New Disinfection Process Criteria"). The guideline requires a 4 log reduction in *Pseudomonas aeruginosa* within 30 seconds of exposure to the disinfection system.

As well as an indicator of how well a disinfection process is performing *P. aeruginosa* can be an important human pathogen being a common cause of pool folliculitis. Patients can present with pruritic follicular, maculopapular, vesicular, or pustular lesions on any part of the body that was immersed in water. Pseudomonal bacteremia produces distinctive skin lesions known as ecthyma gangrenosum. With eye infections, the physical examination reveals lid edema, conjunctival erythema and chemosis, and severe mucopurulent discharge adherent to an underlying corneal ulcer. *P. aeruginosa* is the predominant bacterial pathogen in some cases of external otitis including "swimmer's ear". The bacterium is infrequently found in the normal ear, but often inhabits the external auditory canal in association with injury, maceration, inflammation, or simply wet and humid conditions.

*Pseudomonas* is a gram-negative rod belonging to the family Pseudomonadaceae. Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees. A spa pool provides an ideal environment for *Pseudomonas,* with more than 62% of random cultures showing some positive growth. Human infection is facilitated by dilatation of the pores and superhydration of the stratum corneurn due to the high temperature (Baruchin *et al.* 1996)

## *Methods and Materials*

Two identical spa pools (AAIM QLD Aust.) each with a capacity of 1,500 l were filled with town water (see attached report for typical analysis) and the pool filters left on auto to dissipate the chlorine out of the water (chlorine checked using HACH 2010 spectrophotometer, method 80).

The design of the trial was to have one spa pool the control (untreated) and the other spa pool will have the Enviroswim system (treated). Alkalinity Increaser (Jacks Pool Shop), Hydrochloric acid (BDH, AR) and Sodium chloride (BDH, AR) were added in equal quantities to both spa pools to ensuring that similar and balanced water quality conditions.

The following parameters were recorded for both spas: Conductivity, pH and temperature using a calibrated hand held meter (TPS MC-81). Alkalinity was measured using the titration method (APHA 2320B). Copper and Silver levels were analysed by AA graphite furnace. In addition, TDS and ORP were recorded in the treated spa using in line meters (Milwaukee SM 402 and SM 500 respectively).

The Enviroswim system was switched on prior to inoculation and long enough to establish the desired Oxidation Reduction Potential (ORP) levels for each particular trial (see results below).

For inoculation of spas, a colony of *P. aeruginosa* (ACM 495) on Nutrient agar was aseptically transferred to 100ml of Tryptone Soy Broth and incubated for 24 hrs at 35C. The resulting inoculum was diluted to 2 litres (Schott bottle) of water taken from Spa A just prior to inoculation. Once thoroughly mixed in the Schott bottle, this was the final inoculum which was added to each spa in equal 800 ml portions so as to establish initial levels of *P. aeruginosa* at around of $10^6$ cfu /100ml.

When the 800 ml of inoculum was added to a spa, a timer was started and at the same time the auxiliary pump was activated for 30 sec to provide better mixing than just the circulating pump. Previous trials have established that there is sufficient mixing in 30 secs.

After 30 sec, samples were taken from the spa and the auxiliary pump turned off, with just the circulating pump left on. Further samples were taken at different times to establish levels of reduction in *P. aeruginosa* over time. All samples were taken in 500m sterile bacteriological jars with sodium thiosulphate (Techno-Plas) and processed immediately using the membrane filtration method (APHA 9213E) with mPA-C agar (Amyl). Plates were incubated at 41.5C for 72 hrs and then counted and reported as *P. aeruginosa* cfu/100ml.

*Results*

Trial on 4/2/04

Chlorine in both spas prior to the start of trial was <0.01 mg/l total chlorine.

The water chemistry parameters were recorded for the two Spas 5 min following inoculation and presented in Table 1.

**Table 1 - Water Chemistry in Spa Pools**.

| Spa | pH | Conductivity uS/cm | Temperature °C | Alkalinity mg/l |
|---|---|---|---|---|
| Untreated Spa | 7.1 | 895 | 24 | 85 |
| Enviro-Swim Spa | 7.0 | 880 | 24 | 80 |

The results for the counts of *P. aeruginosa* and the levels of ORP, Copper and Silver in the two Spa pools is presented in Table 2.

**Table 2 - Counts of *P. aeruginosa* and levels of ORP, Copper and Silver.**

| Spa and Time of Sampling | ORP | Cu ug/l | Ag ug/l | *P. aeruginosa* cfu/100ml | *P. aeruginosa* Log/100ml | Log Reduction |
|---|---|---|---|---|---|---|
| Untreated Spa 30 sec | | 11 | 2 | $1.1 \times 10^6$ | 6.041 | |
| Untreated Spa 60 min | | | | $9.9 \times 10^5$ | 5.996 | 0.045 |
| Enviro-swim Spa 30 sec | 760 | 389 | 9 | 14 | 1.146 | **4.895*** |
| Enviro-swim Spa 2 min | 755 | | | < 1 | 0 | 5.996 |
| Enviro-swim Spa 5 min | 748 | | | < 1 | 0 | 5.996 |
| Enviro-swim Spa 60 min | 740 | 417 | 9 | < 1 | 0 | 5.996 |

* The 4.895 log reduction of *P. aeruginosa* after 30 sec in the Enviro-swim Spa exceeds the 4 log reduction required by the NSW Health Department guideline for new disinfection process criteria.

Trial on 24/2/04

Chlorine in both spas prior to the start of trial was <0.01 mg/l total chlorine.

The water chemistry parameters were recorded for the two Spas 5 min following inoculation and presented in Table 3.

**Table 3 - Water Chemistry in Spa Pools**.

| Spa | PH | Conductivity uS/cm | Temperature °C | Alkalinity mg/l |
|---|---|---|---|---|
| Untreated Spa | 7.1 | 770 | 35 | 68 |
| Enviro-Swim Spa | 7.0 | 900 | 35 | 74 |

The results for the counts of *P. aeruginosa* and the levels of ORP, Copper and Silver in the two Spa pools is presented in Table 4.

**Table 4 - Counts of *P. aeruginosa* and levels of ORP, Copper and Silver.**

| Spa and Time of Sampling | ORP | Cu ug/l | Ag ug/l | *P. aeruginosa* cfu/100ml | *P. aeruginosa* Log/100ml | Log Reduction |
|---|---|---|---|---|---|---|
| Untreated Spa 30 sec | | 12 | NA | $7.6 \times 10^6$ | 6.88 | |
| Untreated Spa 60 min | | | | $4.9 \times 10^6$ | 6.69 | 0.19 |
| Enviro-swim Spa 30 sec | 590 | 648^ | NA | 54 | 1.73 | **4.96*** |
| Enviro-swim Spa 2 min | 620 | | | 1 | 0 | 6.88 |
| Enviro-swim Spa 5 min | 609 | | | < 1 | 0 | 6.88 |
| Enviro-swim Spa 60 min | 613 | | | < 1 | 0 | 6.88 |

^ and NA = ICP result only and/or AA tests to be done.

* The 4.895 log reduction of *P. aeruginosa* after 30 sec in the Enviro-swim Spa exceeds the 4 log reduction required by the NSW Health Department guideline for new disinfection process criteria.

***References***

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**Dr PJ Wright**
**(Senior Technical Officer) Date of Report: 06 May 2004**