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Letter to the Editor

Hardness of tap water and *Pseudomonas aeroginosa*

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It is well known that *Pseudomonas aeruginosa* is commonly found in a humid atmosphere such as nebulizers and humidifiers [1–3]. The effect of the hardness of the water on this micro-organism is less known. We wish to report the effect of hardness of tap water used in our hospital on the growth and survival of *P. aeruginosa*. The hardness of water is related to the Ca^{2+} concentration and can be expressed as mmol/l (1 mmol = 56 mg CaO or 100 mg $CaCO_3$/l) or, depending on the country, as °D as used in Germany and the Netherlands (1°D = 10 mg CaO/l) or as °Clark (°C) as used in the United Kingdom (1°C = 14.25 mg $CaCO_3$/l) or as grain per gallon (1 GPG = 17.12 mg $CaCO_3$/l) as used in the United States.

The growth and survival of *P. aeruginosa* (ATCC 27853) in normal tap water and 'decalcified' water was assessed both at room temperature and at 37°C during a 3-week period. Normal tap water in our hospital contained 2.66 mmol Ca^{++}, which is equivalent to 15°D. The hardness of the 'decalcified' water used was 6°D.

From an overnightculture of *P. aeruginosa* suspensions were prepared in normal tap water and in decalcified tap water. Suspensions with a low inoculum size (i.e., 10^2 cfu/ml) were used to test growth, and suspensions containing 10^8 cfu/ml were used for survival experiments. Suspensions of 10^5 cfu/ml were used for both growth and survival.

After inoculation, bottles containing either decalcified or normal tap water were incubated at room temperature or at 37°C. Samples were taken daily during the first week and once a week in the second and third week and cultured semiquantitatively on blood-agar plates using the four quadrants method [4]. Bacterial growth was assesed as follows: i.e., growth with some colonies in the first quadrant was assessed as 10^2 cfu/ml, full growth in the first quadrant as 10^3 cfu/ml and up to full growth in all four quadrants as 10^8 cfu/ml.

After 3 weeks at room temperature the bottles with an initial inoculum of 10^2 cfu/ml in normal tap water contained 10^7 cfu/ml, and bottles with decalcified water contained 10^4 cfu/ml. Incubation at 37°C resulted in a smaller increase in cfu/ml up to 10^4 cfu/ml in normal tap water and to 10^3 cfu/ml in decalcified water. The data in Table 1 show better growth and survival of *P. aeruginosa* in normal tap water than those in 'decalcified' water. The effect was even more pronounced at room temperature than at 37°C; however, the increased Ca^{2+} concentration in tap water also appears to promote growth.

Consequently our results suggest that in hospitals using hard water (more than 10°D) *P. aeruginosa* can survive for a longer period of time than in areas with a low Ca^{2+} concentration in water and that the chance of colonization and infection with this micro-organism will be higher. With this knowledge,

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Table 1
Effect of Ca^{2+} concentration and incubation temperature (decalcified) of tap water on growth and survival of *Pseudomonas aeruginosa*

Inoculum size cfu/ml ($t = 0$)	Incubation temperature	After 3 weeks incubation cfu/ml[a]	
		Normal tap water	"Decalcified" tap water
10^2	room temperature	10^7	10^4
	37°C	10^4	10^3
10^5	room temperature	10^8	10^3
	37°C	10^3	10^4
10^8	room temperature	10^8	10^7
	37°C	10^6	10^3

[a] Mean of 6 experiments.

regular monitoring of the central water supply of our hospital focusses on the presence of *Legionella pneumophilia* as well as *P. aeruginosa* and, in addition, special attention is paid to regular sampling of water from showers and tap water on the wards and in patients' rooms.

A central system for decalcification of the water supply to hospitals and other institutions housing immunocompromised persons (homes for the elderly, day-care centres) is strongly recommended.

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