Suboptimal chlorine treatment of drinking water leads to selection of multidrug-resistant *Pseudomonas aeruginosa*

Richa Shrivastava,[a] R.K. Upreti,[a] S.R. Jain,[a] K.N. Prasad,[b] P.K. Seth,[a] and U.C. Chaturvedi[a,*]

[a] *Biomembrane Division, Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow 226001, India*
[b] *Department of Microbiology, SGPG Institute of Medical Sciences, Lucknow 226001, India*

Received 25 November 2002; received in revised form 11 June 2003; accepted 20 June 2003

Abstract

The present study was undertaken to investigate the spectrum of bacteria present in the River Gomti water before and after chlorination for drinking purposes. We observed that the strains of *Pseudomonas aeruginosa* that survived chlorination on three out of seven occasions were resistant to almost all the antibiotics tested. The chlorine-resistant bacteria had mucoid colonies and grew better at 24°C. All attempts to isolate the plasmid responsible for chlorine resistance were unsuccessful. Laboratory experiments using different strains of the *P. aeruginosa* in distilled water showed that only the resistant strain survived chlorine treatment at a dose of $\leqslant 500\,\mu g/L$. Similar results were obtained when water collected from seven different sites on the River Gomti was treated with graded doses of chlorine. At the higher dose of chlorine, all the bacteria died in 30 min, whereas with lower doses all the bacteria survived. The present study underscores the importance of measuring water chlorine concentrations to assure they are sufficiently high to remove pathogenic bacteria from drinking water. To our knowledge, this is the first report in the literature of the selection of multidrug-resistant bacteria by suboptimal chlorine treatment of water.

Keywords: River water; *Pseudomonas aeruginosa*; Chlorine treatment; Multidrug resistance

1. Introduction

Chlorine is added to drinking water to reduce or eliminate microorganisms, which can be present in water supplies. Most municipal water supplies are chlorinated with chlorine gas. Swimming pools, hot tubs, and the like are usually chlorinated with chlorine-containing substances such as calcium hypochlorite, sodium hypochlorite (bleach), or trichloro-*S*-triazinetrione (commonly known as "trichlor"). In every case, the effectiveness of chlorine as a germicide is a result of chlorine's powerful oxidizing action. The addition of chlorine to drinking water has greatly reduced the risk of waterborne diseases. For more than a century, the safety of drinking water supplies has been greatly improved by chlorine treatment. Still, chlorine remains the most commonly used drinking water disinfectant.

A number of bacteria have been shown to develop resistance to different agents used for the treatment of water, including chlorination (Ridgway and Olson, 1982; Pyle et al., 1994; Le Dantec et al., 2002) and sodium dichloroisocyanurate (D'Auria et al., 1989). Maillard et al. (1998) have reported resistance of *Pseudomonas aeruginosa* PAO1 phage F116 to sodium hypochlorite. Ridgway and Olson (1982) reported that the most sensitive bacteria including *Pseudomonas* spp. are readily killed by chlorine concentrations of $\leqslant 1.0\,mg/L$. Pyle et al. (1994) have isolated a *P. cepacia* strain that has reduced susceptibility to iodine and to chlorination. Stewart et al. (2001) have reported that bacteria in the biofilms of *P. aeruginosa* and *Klebsiella pneumoniae* are highly resistant to killing by both alkaline hypochlorite and chlorosulfamates.

P. aeruginosa is an opportunistic pathogen that is known to cause urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, and a variety of systemic infections, particularly in patients with severe burns, and in cancer

*Corresponding author. Fax: +91-522-2228227.
E-mail address: uchaturvedi@yahoo.com (U.C. Chaturvedi).

0147-6513/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0147-6513(03)00107-6

and AIDS patients who are immunocompromised. This organism grows well at 25–37°C but can grow slowly or at least survive at higher and lower temperatures. Indeed, the ability to grow at 42°C distinguishes it from many other *Pseudomonas* species. In addition to its nutritional versatility, *P. aeruginosa* resists high concentrations of salt, dyes, weak antiseptics, and many commonly used antibiotics. These properties help explain its ubiquitous nature and contribute to its preeminence as a cause of nosocomial infections. *Pseudomonas* species normally inhabit soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They often colonize hospital food, sinks, taps, mops, and respiratory equipment. Spread is from patient to patient by contact with fomites or by ingestion of contaminated food and water (Chastre and Trouillet, 2000; Stewart et al., 2001; Sherman et al., 2001; Shirtliff et al., 2002; Tacconelli et al., 2002). The present study, undertaken to investigate the spectrum of bacteria present in the River Gomti water and during its purification process for drinking, isolated the strains of *P. aeruginosa* that survived suboptimal chlorination; these are the ones that are resistant to almost all the antibiotics tested. This is the first report to our knowledge of the selection of multidrug-resistant bacteria by chlorine treatment of water.

2. Methods and materials

2.1. Collection of water samples

The Gomti River passes through the city of Lucknow and is the main source of drinking water for the city. Water is pumped from the river at Gau Ghat, which is outside the city, and is sent through a pipeline to Lucknow Jal Sansthan, Aishbagh, ~4 km away, where the water is purified and released for drinking. Water was collected at four sites: (1) Gau Ghat, (2) Aishbagh as the water reaches there, (3) after alum treatment, and finally (4) at the exit where the water is released after filtration and chlorination. Water was collected in duplicate sterile containers, at ~30 cm depth, the container facing the direction of the flow of the stream (except at site 4), taking care that water entering the container did not come in contact with the hands. Water samples were collected from site 4 on seven different occasions.

2.2. Chlorine tablets

The chlorine tablets purchased were INSTA-10 (Raman & Weil Pvt. Ltd., Mumbai) containing dichlorosodium isocyanurate equivalent to 25 mg chlorine IP. It was effective for the treatment of 10 L water for 30 min.

2.3. Determination of the most probable number (MPN)

The MPN was determined in the water samples by the five-tube method as described by APHA (1998).

2.4. Isolation of bacteria from water samples

The water samples (500 mL volume) were filtered through a Millipore Filter Assembly with a sterile 0.45-μm membrane filter disk, on which bacteria present in the water sample were retained. Thereafter, the membrane filter disk was aseptically removed by a sterile forceps, cut it into seven or eight pieces, and placed on Petri plates containing the following selective media: MacConkey, deoxycholate citrate, xylose lysine deoxycholate, thiosulfate citrate bile sucrose (TCBS) agar, tetrathionate broth base (TBB), alkaline peptone water (APW), and selenite F-broth (SFB). After 30–45 min the filter paper was removed from the plate with the help of sterile forceps, and the plates were streaked appropriately. The broth tubes were vortexed, incubated at 37°C for 6–8 h, and then the culture was streaked on an appropriate plate of APW tubes. All other cultures were incubated at 37°C for 24–48 h and were then examined for bacterial growth and colony characteristics. Gram-stained smears were examined. The isolates were identified by different biochemical tests, according to the criteria given in *Bergey's Manual of Systematic Bacteriology*.

2.5. Antibiotic sensitivity by disk method

All the strains of *P. aeruginosa* isolated from the water samples were tested for antibiotic sensitivity following the National Committee for Clinical Laboratory Standard (NCCL) disk diffusion method. The following antibiotic disks were used: gentamicin (10 μg), amikacin (30 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), and nalidixic acid (30 μg). The different strains of *P. aeruginosa* isolated from water and the control strain NCTC 10662 were tested. A control plate without antibiotics was included in each series.

2.6. Screening for plasmid

The bacterial cells were grown overnight in LB medium at 24°C and 37°C in a shaker at 100 rpm. The cells were pelleted at 8000*g* for 10 min at 4°C. DNA was extracted from the bacteria using Concert high-purity plasmid purification systems Midi Prep protocol (Life Technologies Inc, USA) Qiagen Maxi Plasmid Prep protocol (Qiagen, Germany) as per manufacturer's instructions. Plasmid DNA was also isolated by the method of Kado and Liu (1981). Agarose gel electrophoresis was carried out using 0.7% agarose with a low-salt buffer system composed of 40 mM Tris–acetate and

1 mM sodium EDTA. Molecular weight ladders λ *Hin*dIII (MBI, Fermentas, Inc) were used. The bands were located by staining with ethidium bromide.

2.7. Statistical analysis

Student's *t*-test was used for statistical evaluation of the data. A P value of <0.05 was considered significant.

3. Results

3.1. Bacterial profile in the river water at different sites

A profile of the bacteria present in the river water at different sites was obtained by estimation of MPN, as presented in Table 1. The findings show that MPN in crude river water was 4600/100 mL, whereas that in purified water ready for consumption was 4/100 mL. Various types of bacteria present in the water at different sites are shown in Table 1. *P. aeruginosa* was present at all the four sites, even after chlorination. We decided to study the strains of *P. aeruginosa* isolated from the different sites in detail. It was observed that the *Pseudomonas* strain isolated from the river water was sensitive to cefotaxime, ciprofloxacin, norfloxacin, amikacin, and gentamicin (therefore labeled as "sensitive strain") and was resistant to nalidixic acid as shown by the disk method, whereas the strain isolated from the chlorinated water was resistant to all the above antibiotics ("resistant strain") except norfloxacin and ciprofloxacin. This phenomenon was observed in three out of seven repeated samples tested. On careful enquiry we realized that on the three occasions in question the supply of the chlorine gas might not have been appropriate. This led to detailed investigation in the laboratory as described later.

Table 1
Bacterial profile in the water from Gomti River to Aishbagh water filtration plant

Sites	MPN index (100 mL)[a]	Bacteria isolated
1. Gau Ghat (the river)	4600	*Escherichia coli* *Enterobacter* spp. *P. aeruginosa* *Citrobacter freundi* *Aeromonas hydrophila* *Enterococcus* spp.
2. Untreated water at Aishbagh	5000	*E. coli* *P. aeruginosa* *C. freundi* *Klebsiella pneumoniae*
3. After alum treatment	3000	*Proteus mirabilis* *Enterococcus faecalis* *Aeromonas* spp. *E. coli* *P. aeruginosa*
4. After filtration and chlorination	4	*P. aeruginosa*

[a] MPN, most probable number.

3.2. Selection of multidrug-resistant P. aeruginosa by chlorine treatment of water inoculated with known bacteria

We decided to attempt to determine whether chlorine treatment of water in the laboratory could select for the resistant strains of *P. aeruginosa*. Equal amounts (200 µL containing 400 colony-forming units) of the "sensitive" or the "resistant" strains of *P. aeruginosa* were inoculated in 500 mL of sterile distilled water, which was then treated with various doses of chlorine at room temperature (25–27°C). The control group remained untreated. After 30 min the entire volume of water was passed through the Millipore membrane and then cultured on media as described earlier. At 24 and 48 h, the cultures were examined and the colony-forming units were counted. The colonies were tested for the drug-resistant strains. The mean findings from four experiments summarized in Table 2 show that a dose >500 µg/L of chlorine killed all the bacteria inoculated. The difference in the colony count was significantly ($P\leqslant 0.001$) higher for the resistant strain at higher concentrations of chlorine (Table 2).

3.3. Selection of multidrug-resistant P. aeruginosa by chlorine treatment of Gomti River water

Water was collected from seven locations on the River Gomti: (1) From Gau Ghat (upstream), which is just

Table 2
Selection of multidrug-resistant *P. aeruginosa* by chlorine treatment of water inoculated with known "sensitive" or the "resistant" strains of *P. aeruginosa*

Chlorine concentration (µg/L)	Colony-forming units	
	"Sensitive" strain	"Resistant" strain
1000	0	0
500	0	90±5
200	10±1	100±9
100	16±3	170±21
20	150±12	200±14
10	200±18	220±18
5	270±25	290±25
2.5	270±22	300±22
1.7	300±27	320±27
1.25	300±19	330±21
1	300±22	330±25
0.75	320±20	330±17
Control	340±25	335±28

outside the city of Lucknow in the North, to across the city at (2) Hardinge's bridge, (3) Shaheed Smarak, (4) Nishatgunj bridge, (5) Gomti Barrage, (6) Pipraghat, and (7) Malhaur, which is outside the city in the South. The distance from Gau Ghat to Malhaur is 18 km. *P. aeruginosa* were isolated from the water at all seven sites. After treatment of a 1-L water sample from each site with 500 µg/L of chlorine for 30 min, it was filtered and bacteria isolated. The data presented in Table 3 show that *P. aeruginosa* were isolated from sites 1, 5, and 7 after chlorine treatment and were resistant to multiple drugs.

Table 3
Selection of multidrug-resistant *P. aeruginosa* by chlorine treatment of the Gomti River water

Sites	Before chlorine treatment	After chlorine treatment
1. Gau Ghat	*E. coli* *Enterobacter* spp. *P. aeruginosa* *Citrobacter freundi* *Aeromonas hydrophila* *Enterococcus* spp.	*P. aeruginosa*[a]
2. Hardinge's bridge	*E. coli* *Klebsiella* spp. *P. aeruginosa* *C. freundi*	Nil
3. Shaheed Smarak	*Proteus mirabilis* *P. aeruginosa* *C. freundi*	Nil
4. Nishatgunj bridge	*Klebsiella* spp. *P. aeruginosa* *E. coli* *C. freundi*	Nil
5. Gomti Barrage	*E. coli* *Klebsiella* spp. *Aeromonas* spp. *P. aeruginosa* *C. freundi*	*P. aeruginosa*[a]
6. Pipraghat	*Aeromonas* spp. *E. coli* *Klebsiella* spp. *P. aeruginosa*	Nil
7. Malhaur	*Klebsiella* spp. *P. aeruginosa* *C. freundi*	*P. aeruginosa*[a]

[a] Multi-drug resistant.

3.4. Antibiotic sensitivity

Sensitivity to different antibiotics was estimated using different strains of *P. aeruginosa* isolated from water and the standard control NCTC 10662 strain. Table 4 summarizes the antibiotic sensitivity profiles of the different strains.

3.5. Screening for plasmids

The results of agarose gel electrophoresis in Fig. 1 show identical bands at the 22-kb position in both strains of *P. aeruginosa,* the one isolated before filtration and chlorine treatment and the other after the treatment. There was no extra band in the chlorine-resistant bacteria, even when grown in the presence of the antibiotic gentamicin.

4. Discussion

The most important finding of the present study is the demonstration of the selection of multidrug-resistant *P. aeruginosa* in river water treated with a suboptimal concentration of chlorine. This finding was confirmed in the laboratory, and the suboptimal dose of chlorine required for such selection was established. Furthermore, this dose of chlorine could select the multidrug-resistant *P. aeruginosa* from the untreated river water at three sites on the Gomti River (Table 3). A number of

Table 4
Antibiotic sensitivity of the different strains of *P. aeruginosa*

	Range of antibiotics tested (µg)					
Site of isolation	Nalidixic acid (30)	Gentamicin (10)	Ciprofloxacin (5)	Cefotaxime (30)	Amikacin (30)	Norfloxacin (10)
1. Gau Ghat	R[a]	R	S[b]	R	R	S
2. Hardinge's bridge	R	S	S	S	S	S
3. Shaheed Smarak	R	S	S	R	S	S
4. Nishatgunj bridge	R	S	S	R	S	S
5. Gomti Barrage	R	R	S	R	R	S
6. Pipraghat	R	R	S	S	S	S
7. Malhaur	R	R	S	R	R	S
1A. Aishbagh	R	S	S	S	S	S
1C. After chlorination	R	R	S	R	R	S

[a] Resistant.
[b] Sensitive.

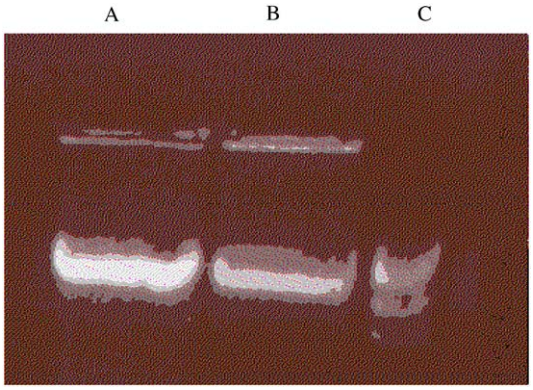

Fig. 1. Agarose gel electrophoresis of the plasmid DNA purified from the *P. aeruginosa* isolated from the water of River Gomti before (A) and after (B) filtration and chlorine treatment. (C) Molecular weight marker.

bacteria have been shown to develop resistance to different agents used for the disinfection of water, for example, chlorination. Ridgway and Olson (1982) compared the relative chlorine sensitivities of bacteria isolated from chlorinated and unchlorinated drinking water distribution systems and found that bacteria from the chlorinated system were more resistant to both the combined and free forms of chlorine than those from the unchlorinated system, suggesting that there may be selection for more chlorine-tolerant microorganisms in chlorinated waters. Hingst et al. (1995) searched for bacteria with an elevated resistance to antiseptics and disinfectants, including formaldehydes, biguanides, quaternary ammonium compounds (QACs), phenols, and halogens. The maximal tolerated concentrations to these antimicrobial agents in samples of treated wastewater effluent show a considerably higher prevalence of bacteria resistant to formaldehyde, chlorhexidine, and QACs. The highest levels of resistance are found in *P. aeruginosa* (Hingst et al., 1995). Le Dantec et al. (2002) have reported that *Mycobacterium gordonae*, an atypical mycobacterium isolated from a water distribution system, is more resistant to chlorine in low-nutrient media, such as those encountered in water, and that an increase in temperature (from 4°C to 25°C) and a decrease in pH result in better inactivation. Russell (2002) has discussed the introduction of biocides, including chlorine and other chlorine-releasing agents, into clinical practice, and their impact on antibiotic-resistant bacteria. Of the different biocides, cationic agents (QACs, chlorhexidine, diamidines, acridines) and triclosan have been implicated in the selection and persistence of bacterial strains with low-level antibiotic resistance. In contrast, our findings showed selection of multidrug-resistant *P. aeruginosa.*

The present study has demonstrated that the untreated water of the River Gomti contains a variety of pathogenic and potentially pathogenic bacteria, including *P. aeruginosa.* A similar variety of bacteria have been shown to be present in river waters in several different countries (Hingst et al., 1995; Al-Jebouri, 1985; Sokari et al., 1988; De et al., 1993; Campeau et al., 1996). Fernandez-Alvarez et al. (1991) evaluated the microbiological quality of the River Riato in Spain and reported that the counts of fecal indicators increase considerably when cattle are allowed to roam free. In contrast, Aoi et al. (2000) have shown that *P. aeruginosa* strains isolated from river water originates from the environment of human activity and not from wildlife or domestic animals. The presence of different forms of pathogenic and potentially pathogenic bacteria seems to be due to brisk activity of humans and animals around the River Gomti. To clarify the relationship between water pollution and bacterial flora in rivers, Wada (1993) collected samples of river water at 11 stations of the Chikuma-Sai River system in Japan from April 1985 to March 1986 and suggested that bacterial populations, including *Pseudomonas* and the coliform group, increased in direct proportion to the extent to which organic substances exceeded the capacity for self-purification of the river.

The findings further showed that the MPN of the Gomti River water at the seven different sites was 4.6×10^3–4.6×10^6/100 mL (full data not shown here), a similar result to that reported by Fernandez-Alvarez et al. (1991) in the River Riato in Spain. After purification and chlorination, the water that is released for drinking had a MPN index of 4/100 mL of water, which is of ''intermediate'' quality grade according to APHA (1998) criteria. Our study shows that the only bacteria that survived chlorine treatment of drinking water on three out of seven occasions were *P. aeruginosa* and that these bacteria were resistant to all the antibiotics tested. *P. aeruginosa* is notorious for its resistance to antibiotics and is therefore a particularly dangerous and dreaded pathogen. The bacterium's natural resistance to many antibiotics is due to the permeability barrier afforded by its outer membrane lipopolysaccharides. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Because its natural habitat is the soil, living in association with the bacilli, actinomycetes, and molds, it has developed resistance to a variety of their naturally occurring antibiotics. Moreover, *Pseudomonas* spp. maintain antibiotic resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of the bacterial processes of transduction and conjugation. Only a few antibiotics are effective against *Pseudomonas* spp., including fluoroquinolones, gentamicin, and imipenem, and even these antibiotics are not effective against all strains (reviewed by Kato et al., 2001).

Campeau et al. (1996) examined Gram-negative bacilli in the Detroit River (USA) for resistance to agents of interest and found that lactose-nonfermenting isolates demonstrated resistance to six of nine antimicrobial agents, when tested by a paper disk procedure. The most common group of resistant bacteria was *P. fluorescens*. A comparison of protein profiles produced by polyacrylamide gel electrophoresis indicated that there was variation between *P. fluorescens* strains, showing the same multiple-drug resistance (Campeau et al., 1996). Kato et al. (2001) studied the resistance of *P. aeruginosa* to meropenem, imipenem, panipenem, piperacillin, ceftazidime, cefozopran, cefoperazone, sulbactam/cefoperazone, amikacin, and tobramycin, and they also investigated cross-resistance profiles. Overall, 8.3% of isolates were imipenem-resistant and 4.6% were ceftazidime-resistant. However, the incidence of antibiotic-resistant *P. aeruginosa* was distinctly different at each location. Our findings presented in Table 3 confirm this observation.

Moreover, in our plasmid study we have used plasmid isolation protocols that can screen all types and sizes of plasmid DNA. However, we did not find the one responsible for chlorine resistance, even after growing the bacteria in the presence of the antibiotic gentamicin. It is likely that the genes responsible for chlorine resistance are not plasmid-encoded. The exact molecular basis (that is, genes involved and genetic responses specific to chlorine exposure) is not known. It was also observed that the colonies of the chlorine-resistant *P. aeruginosa* were mucoid and grew better at 24°C. Mucoid strains of *P. aeruginosa* are characterized by an overproduction of extracellular alginate. It has been suggested that alginate-containing slime may contribute to survival of these bacteria in chlorinated water systems (Grobe et al., 2001). This phenomenon indicates involvement of multiple genes and pathways (reviewed by Dussart et al., 2003).

Thus, the most important finding of the present study is the demonstration that selection of multidrug-resistant *P. aeruginosa* occurred in river water treated with suboptimal concentrations of chlorine. The present study underscores the importance of measuring chlorine concentrations in water in ensuring that specified standards are maintained and that low chlorine levels do not result in survival in treated water of pathogenic bacteria.

Acknowledgments

We thank R. Shanker for advice on plasmid demonstration. U.C. Chaturvedi is an Emeritus Scientist of the Council of Scientific and Industrial Research, New Delhi (Project No. 21(0478)/00/EMR-II).

References

Al-Jebouri, M.M., 1985. A note on antibiotic resistance in the bacterial flora of raw sewage and sewage-polluted river Tigris in Mosul, Iraq. J. Appl. Bacteriol. 58, 401–406.

Aoi, Y., Nakata, H., Kida, H., 2000. Isolation of *Pseudomonas aeruginosa* from Ushubetsu river water in Hokkaido, Japan. Jpn. J. Vet. Res. 48, 29–34.

American Public Health Association (APHA), 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. American Public Health Association, Washington, DC, pp. 9.1–9.113.

Campeau, R.C., Gulli, L.F., Graves, J.F., 1996. Drug resistance in Detroit River Gram-negative bacilli. Microbios 88, 205–212.

Chastre, J., Trouillet, J.L., 2000. Problem pathogens (*Pseudomonas aeruginosa* and *Acinetobacter*). Semin. Respir. Infect. 15, 287–298.

D'Auria, F.D., Simonetti, G., Strippoli, V., 1989. Antimicrobial activity exerted by sodium dichloroisocyanurate. Ann. Ig. 1, 1445–1458.

De, A., Sen, P.C., Tewari, I.C., 1993. Enteropathogenic bacteria in River Ganges in Varanasi. Indian J. Pathol. Microbiol. 36, 425–432.

Dussart, L., Dupont, J-P., Zinmerlin, I., Lacroix, M., Saiter, J.M., Junter, G.A., Jouenne, T., 2003. Occurrence of sessile *Pseudomonas oryzihabitans* from a karstified chalk aquifer. Water Res. 37, 1593–1600.

Fernandez-Alvarez, R.M., Carballo-Cuervo, S., de la Rosa-Jorge, M.C., Rodriguez-de Lecea, J., 1991. The influence of agricultural run-off on bacterial populations in a river. J. Appl. Bacteriol. 70, 437–442.

Grobe, S., Wingender, J., Flemming, H.C., 2001. Capability of mucoid *Pseudomonas aeruginosa* to survive in chlorinated water. Int. J. Hyg. Environ. Health 204, 139–142.

Hingst, V., Klippel, K.M., Sonntag, H.G., 1995. Epidemiology of microbial resistance to biocides. Zentralbl. Hyg. Umweltmed. 197, 232–251.

Kado, C.I., Liu, S.T., 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145, 1365–1373.

Kato, K., Iwai, S., Kumasaka, K., Horikoshi, A., Inada, S., Inamatsu, T., Ono, Y., Nishiya, H., Hanatani, Y., Narita, T., Sekino, H., Hayashi, I., 2001. Survey of antibiotic resistance in *Pseudomonas aeruginosa* by the Tokyo Johoku association of *Pseudomonas* studies. J. Infect. Chemother. 7, 258–262.

Le Dantec, C., Duguet, J.P., Montiel, A., Dumoutier, N., Dubrou, S., Vincent, V., 2002. Chlorine disinfection of atypical mycobacteria isolated from a water distribution system. Appl. Environ. Microbiol. 68, 1025–1032.

Maillard, J.Y., Hann, A.C., Perrin, R., 1998. Resistance of *Pseudomonas aeruginosa* PAO1 phage F116 to sodium hypochlorite. J. Appl. Microbiol. 85, 799–806.

Pyle, B.H., Watters, S.K., McFeters, G.A., 1994. Physiological aspects of disinfection resistance in *Pseudomonas cepacia*. J. Appl. Bacteriol. 76, 142–148.

Ridgway, H.F., Olson, B.H., 1982. Chlorine resistance patterns of bacteria from two drinking water distribution systems. Appl. Environ. Microbiol. 44, 972–987.

Russell, A.D., 2002. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. J. Appl. Microbiol. 92 (Suppl), 121S–135S.

Sherman, N.E., Stefansson, B., Fox, J.W., Goldberg, J.B., 2001. *Pseudomonas aeruginosa* and a proteomic approach to bacterial pathogenesis. Dis. Markers 17, 285–293.

Shirtliff, M., Mader, J., Camper, A., 2002. Molecular interactions in biofilms. Chem. Biol. 9, 859.

Sokari, T.G., Ibiebele, D.D., Ottih, R.M., 1988. Antibiotic resistance among coliforms and *Pseudomonas* spp. from bodies of water around port Harcourt, Nigeria. J. Appl. Bacteriol. 64, 355–359.

Stewart, P.S., Rayner, J., Roe, F., Rees, W.M., 2001. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. J. Appl. Microbiol. 91, 525–532.

Tacconelli, E., Tumbarello, M., Bertagnolio, S., Citton, R., Spanu, T., Fadda, G., Cauda, R., 2002. Multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections: analysis of trends in prevalence and epidemiology. Emerg. Infect. Dis. 8, 220–221.

Wada, M., 1993. Relationship between water pollution and bacterial flora in river water. Nippon Eiseigaku Zasshi 48, 707–720.