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Selection of Antibiotic-Resistant Standard Plate Count Bacteria During Water Treatment†

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Standard plate count (SPC) bacteria were isolated from a drinking-water treatment facility and from the river supplying the facility. All isolates were identified and tested for their resistance to six antibiotics to determine if drug-resistant bacteria were selected for as a consequence of water treatment. Among the isolates surviving our test procedures, there was a significant selection ($P < 0.05$) of gram-negative SPC organisms resistant to two or more of the test antibiotics. These bacteria were isolated from the flash mix tank, where chlorine, alum, and lime are added to the water. Streptomycin resistance in particular was more frequent in this population as compared with bacteria in the untreated river water ($P < 0.01$). SPC bacteria from the clear well, which is a tank holding the finished drinking water at the treatment facility, were also more frequently antibiotic resistant than were the respective river water populations. When 15.8 and 18.2% of the river water bacteria were multiply antibiotic resistant, 57.1 and 43.5%, respectively, of the SPC bacteria in the clear well were multiply antibiotic resistant. Selection for bacteria exhibiting resistance to streptomycin was achieved by chlorinating river water in the laboratory. We concluded that the selective factors operating in the aquatic environment of a water treatment facility can act to increase the proportion of antibiotic-resistant members of the SPC bacterial population in treated drinking water.

The frequent occurrence of sizable numbers of standard plate count (SPC) bacteria in drinking water has recently led to a closer examination and assessment of the significance of these organisms (8, 12, 15). There is concern that several SPC genera may pose a hazard to public health (6, 7). Also, high numbers of SPC bacteria have been found to interfere with the detection of coliforms in water and frequently must be reckoned with in the assessment of water quality (5). Some investigators point to the SPC as a potentially more reliable indicator of drinking water quality than the coliform index (5, 13). Others have discussed the usefulness of the SPC as a means to assess the ongoing efficiency of treatment facilities (10, 11).

Recently, we reported another feature of bacteria within the SPC populations from several distribution water systems in Oregon: the occurrence of high frequencies of antibiotic-resistant organisms (3). The percentage of multiply antibiotic-resistant (MAR) bacteria was found to be significantly greater among isolates from distribution water samples than that of bacteria in corresponding untreated source waters. We propose that this occurred as a consequence of selective mechanisms operating during water treatment or in the distribution pipelines or both. In the current report, attention is focused on the role of the water treatment facility in this selection of antibiotic-resistant bacteria.

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MATERIALS AND METHODS

River water and municipal treatment facility used for sampling. River water samples were taken from a large use-reuse river in Oregon. The flash mix-treated and clear well water samples were taken from a water treatment plant that uses this river for its source water. The facility has a capacity of 79×10^6 liters per day and supplies a population of about 40,000. Figure 1 shows the essential features of the plant's design. Raw water is first pumped to the flash mix tank (dimensions, 3.7 by 3.1 by 3.4 m), where it is mixed with chlorine (free residual, ≈1.5 mg/liter), alum (aluminum sulfate at 16 to 20 mg/liter), and lime (added in batch quantities to give a pH of ≈6.7 to 6.9). The water next flows to the four flocculation basins (dimensions, 13.4 by 4.9 by 2.4 m). Rotating paddles agitate the water and ensure maximum alum floc formation. Next, the floc is allowed to settle in the four sedimentation basins (dimensions, 27.4 by 4.9 by 4.9 m). The water is then passed through four filters (dimensions, 16.7 by 6.7 by 3.7 m) that contain 46 cm of anthracite coal overlaying 23 cm of silica sand, which is layered on 8 cm of garnet sand. Each filter can accommodate a maximum flow of 244 liters/m^2 per min, which is equivalent to 19×10^6 liters per day. The filtered water is pumped to a tank, called the clear well, at the treatment plant. Just before entering the clear well, the

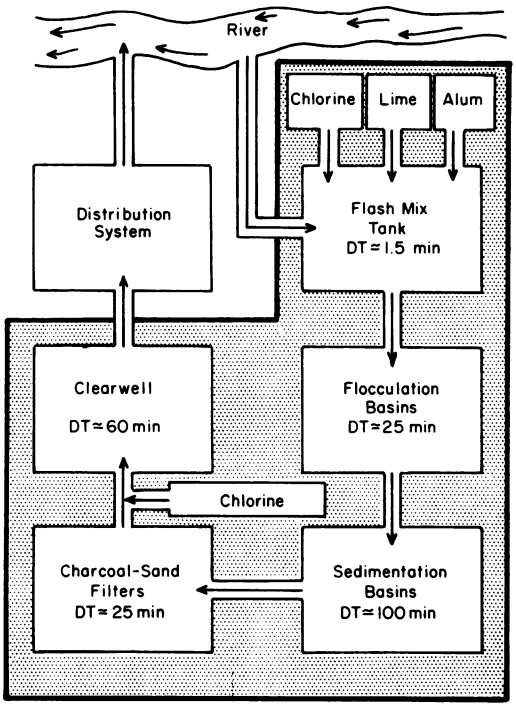

FIG. 1. Diagram of water treatment plant. Capacity flow rate is 79×10^6 liters per day. Detention time (DT) is calculated for the facility operating at 38×10^6 liters per day. The detention times in pipes connecting tanks are all less than 1 min.

water is again chlorinated to bring the free residual chlorine to 0.7 mg/liter. The clear well can hold 1.5×10^6 liters of water. The flash mix-treated waters were obtained from a flocculation basin at the inlet from the flash mix tank. Clear well samples were taken from a constantly running faucet in the treatment facility laboratory, which is connected directly to the clear well.

Collection of samples. Water samples were obtained from the river on 23 March, 11 May, and 8 June 1981. On 23 March and 11 May, the flash mix-treated water was sampled. On 11 May and 8 June, the clear well was sampled. Standard methodology (2) was used as described previously (3).

Enumeration of SPC bacteria. Water samples were filtered through GN-6 Gelman filters with a pore size of 0.45 μm. SPC densities were obtained from filters placed on membrane-SPC (M-SPC) agar (18), incubated for 48 h at 35°C, and examined under ×15 magnification.

Purification of isolates. A total of 200 to 500 colonies were picked at random from the filters and streaked onto glucose-free tryptic soy agar (Difco Laboratories, Detroit, Mich.) containing 0.3% added yeast extract (Difco). After incubation for 24 to 48 h at 35°C, a single colony of each isolate was picked, again streaked onto tryptic soy agar-yeast extract, and identified.

Identification. Isolates were identified by a method similar to that of LeChevallier et al. (8). Isolates were placed into genera or groups on the basis of cell and colonial morphology, Gram stain, motility, oxidase test, and glucose fermentation and oxidation. The API 20E system was used for generic identification of enteric organisms.

Antibiotic resistance testing. A variation of the replica plate procedure already described (3) was used to detect drug-resistant organisms. Purified isolates were picked from tryptic soy agar-yeast extract plates and inoculated into Mueller-Hinton broth (Difco) in test tubes (75 by 100 mm). After 24 h of incubation at 35°C, these cultures were diluted to about 10^5 colony-forming units (CFU)/ml with fresh Mueller-Hinton broth. A sterile multipoint inoculation device holding 38 3-mm-diameter stainless steel rods was used to replicate these cultures to the surfaces of plates containing Mueller-Hinton agar supplemented with antibiotics. Plates of Mueller-Hinton agar lacking antibiotics were inoculated first and last as controls for growth. The presence or absence of growth was noted after 18 h of incubation at 35°C, in accordance with the standard procedure for antibiotic resistance testing (20). The six antibiotics used were disodium carbenicillin (350 μg/ml), chloramphenicol (25 μg/ml), kanamycin sulfate (25 μg/ml), streptomycin sulfate (15 μg/ml), sulfanilamide (350 μg/ml), and tetracycline hydrochloride (12 μg/ml). Carbenicillin (Geopen) was purchased from Pfizer Inc., New York. The other five drugs were obtained from Sigma Chemical Co., St. Louis, Mo. Antibiotic-containing media were used within 2 days of preparation. Two procedures for antibiotic resistance testing of isolates from laboratory-chlorinated samples were used. One method involved the use of a needle replicating device, media, and an incubatior time of 48 h, as described in an earlier paper (3). The second method is described above and was used for one laboratory chlorination experiment performed on 23 March 1981. The differences between the two methods lay in the design of the replicating device (stainless steel rods versus nichrome wire needles) and the incubation times (18 versus 48 h).

Laboratory chlorination of river water. A 500-ml volume of river water was mixed with 1.5 ml of a stock calcium hypochlorite solution [containing 820 mg of $Ca(OCl)_2$ per liter] to give an experimentally determined free residual chlorine level of 1.5 mg/liter. After 60 min at 10°C with mixing at 120 rpm, 0.5 ml of 10% sodium thiosulfate was added to the chlorinated water, volumes were filtered, and the filters were placed onto M-SPC agar. The free residual chlorine level, exposure time, and incubation temperature were selected to approximate conditions in the treatment facility.

RESULTS

The SPC and free residual chlorine level for each water sample are summarized in Table 1. There were 3,800- and 640-fold decreases in the SPC CFU per milliliter when bacteria were exposed to the flash mix tank environment on 23 March and 11 May, respectively. Compared with the river waters sampled on 11 May and 8 June, the SPCs of the clear well samples were around 600,000-fold lower.

Table 1 also summarizes the percentages of isolates picked from filters which subsequently survived purification, identification, and testing for antibiotic resistance. For example, on 8

TABLE 1. Free residual chlorine levels and SPCs of river and treatment plant water samples and percentages of isolates surviving laboratory handling

Date (mo/day/yr)	Water used as sample source	FRC[a] (mg/liter)	SPC (CFU/ml)	No. (%[b]) of isolates picked from filters on M-SPC agar
3/23/81	River	0	16,000	517 (19.0)
	Flash mix treated	0.6	4.2	208 (45.7)
5/11/81	River	0	5,400	457 (44.2)
	Flash mix treated	0.3	8.4	269 (70.3)
	Clear well	0.7	0.009	106 (79.2)
6/8/81	River	0	18,500	514 (41.6)
	Clear well	0.8	0.03	258 (35.7)

[a] FRC, Free residual chlorine.

[b] Numbers in parentheses represent percentages of isolates picked from filters on M-SPC agar that survived purification, identification, and antibiotic resistance testing.

June, of 514 river water isolates and 258 clear well isolates picked, 41.6 and 35.7%, respectively, survived purification and testing procedures. Data shown in Table 2 and Fig. 2 through 5 are based on the surviving populations. The total number of survivors analyzed from each sample is indicated in the diagrams. This loss in viability is known to be a common occurrence by others who work with environmental organisms (D. J. Reasoner and E. E. Geldreich, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, N27, p. 177).

In matched samples collected on the same date, the bacterial populations surviving laboratory handling and testing obtained from both the flash mix tank and clear well water samples were markedly different in their taxonomic compositions as compared with those of the populations in untreated river waters. The frequencies of the specific types of bacteria and the total numbers identified and tested for antibiotic resistance from each sample are shown in Table 2. The predominant organisms observed in untreated river water samples were gram-negative, weakly fermentative or nonfermentative types of the *Moraxella*-like *Flavobacterium* and *Pseudomonas-Alcaligenes* groups and *Microcyclus*. These organisms comprised over 70% of the populations examined in each of the three river water samples. The remaining types in the populations included less than 7% gram-negative, fermentative rods and about 10 to 25% gram-positive bacteria. The most outstanding difference between bacterial taxonomic groups in the untreated river water and flash mix-treated waters was the predominance of gram-positive rods, which comprised about 80% of the bacteria in the latter. About 12% of the populations were gram-negative rods in the two flash mix-treated samples analyzed. We also found the clear well water samples to have distinctly different populations as compared with both the flash mix and river water samples. The *Pseudomonas-Alcaligenes* group comprised about 36% of the clear well populations. Around 12% were in the *Moraxella*-like *Flavobacterium* group, and 35 to 40% were gram-positive rods.

Associated with the fluctuations in population composition at various treatment stages were variations in the kinds and percentages of antibiotic resistance phenotypes of the isolates. Figures 2, 3, and 4 show the proportions of bacteria from river and treated waters that were sensitive to all six test antibiotics, resistant to one of the drugs, or MAR. A statistical comparison of all MAR bacteria in the river water and flash mix-treated samples obtained on 23 March (Fig. 2) and 11 May (Fig. 3) indicated no significant differences at the 5% confidence level. However, the frequencies of the gram-negative subset of the MAR isolates were significantly higher in the flash mix-treated water on both 23 March ($P = 0.03$) and on 11 May ($P < 0.01$) compared with the gram-negative MAR bacteria in the respective raw-water samples. The MAR gram-positive subset of the flash mix tank population on 23 March was not significantly different from the raw water, but on 11 May, the proportion of the MAR gram-positive bacteria was lower in the flash mix-treated water as compared with that in the river water ($P < 0.01$).

The differences in frequencies of all MAR bacteria in river water and clear well samples were highly significant on both 11 May and 8 June (Fig. 3 and 4; $P < 0.01$ in both cases). On 11 May, 11.6% (20 of 173) of the gram-negative isolates from the river water were MAR compared with 80.0% (40 of 50) in the clear well ($P < 0.01$). Also on 11 May, the *Pseudomonas-Alcaligenes* subset of the gram-negative population showed this trend when 26 of 30 (86.7%) of these isolates from the clear well were MAR and 6 of 14 (42.9%) isolates from the river water were MAR ($P < 0.01$). The percentages of MAR gram-positive rods in the clear well samples

obtained on 11 May and 8 June were not different at the 5% confidence level as compared with those in river water. On 8 June, the frequencies of MAR gram-negative isolates in river and clear well samples were not significantly different at the 5% level (river water, 32 of 188 [17.0%] MAR; clear well, 10 of 47 [21.3%] MAR). However, the MAR gram-positive rods increased from 27.0% (7 of 26) to 71.8% (28 of 39; $P < 0.01$) on this date.

Figure 5 shows the frequencies of isolates obtained on 11 May that were resistant to each of the specific test antibiotics. There was a noticeable increase in the percentage of organisms in flash mix-treated water as compared with those in raw water that were resistant to carbenicillin ($P < 0.01$), streptomycin ($P < 0.01$), and kanamycin ($P = 0.03$). This was the case even though there was not a significant difference in the overall frequencies of all MAR isolates (Fig. 3). Furthermore, on this date, the clear well contained increased frequencies of all bacteria resistant to carbenicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline as compared with the untreated river water, primarily owing to a significant increase in the frequencies of resistance phenotypes among the gram-negative members of the clear well population. The gram-positive bacteria in both the flash mix and clear well samples obtained on 11 May exhibited lower frequencies of resistance to kanamycin ($P < 0.01$) and tetracycline ($P < 0.01$) as compared with the respective raw water gram-positive isolates.

The type of data presented in Fig. 5 can also be briefly summarized for the 23 March river and flash mix-treated samples. The significant changes were an increase in resistance to streptomycin (12 of 74 [16.2%] to 7 of 12 [58.3%], $P < 0.01$) and a drop in resistance to sulfanilamide (57 of 74 [77.0%] to 5 of 12 [41.7%], $P < 0.01$) among the gram-negative subsets of the populations. This same general information can also be summarized for the 8 June sampling comparing river and clear well water (data not shown). There were significantly higher levels of resistance to carbenicillin, streptomycin, and sulfanilamide ($P < 0.01$ in all cases) found among bacteria from the clear well. This correlated with the greater percentage of overall MAR bacteria in the clear well (43.5%) compared with that from the river water (18.2%; Fig. 4). Resistance to tetracycline was higher among the gram-negative isolates from the clear well (10.6%) than it was among the river water population (1.6%; $P < 0.01$). The frequency of gram-negative, sulfanilamide-resistant organisms decreased from 46.8 to 29.8% ($P = 0.03$). It was the gram-positive rod subset of the clear well population on 8 June which comprised the higher proportion of MAR bacteria observed in the sample. This was associated with significantly more gram-positive isolates resistant to carbenicillin ($P < 0.01$), streptomycin ($P = 0.03$), and sulfanilamide ($P < 0.01$). Both chloramphenicol ($P = 0.03$) and kanamycin ($P < 0.01$) resistances were less frequent among gram-positive rods from the clear well on 8 June.

In addition to the analyses of bacteria from river and flash mix-treated waters on 23 March, we also studied those isolates that survived laboratory chlorination of the river water sampled on this date. Chlorine was added to the water to give a free residual level of 1.5 mg/liter. After 1 h, the SPC population decreased 800-fold to 20 CFU/ml. Survivors were purified, identified, and tested for their resistance to the six test antibiotics. This experiment paralleled the analyses of bacteria from water treated in the flash mix tank at the treatment facility on the same day (see Table 2 and Fig. 2). Laboratory chlorination served as a means to separate the effects of chlorine from other physicochemical factors in the flash mix tank that could be influencing changes in the microbial population. A statistical comparison of the taxonomic groups in this laboratory-chlorinated sample and in the flash mix-treated sample showed no major differences based on $P \leq 0.05$. In regard to the frequencies of specific antibiotic resistance characters, streptomycin resistance was significantly more common ($P < 0.01$) among gram-negative isolates from both flash mix-treated and laboratory-chlorinated water samples as compared with gram-negative isolates from raw water.

The selection for a specific antibiotic resistance phenotype as a consequence of chlorinating raw-water samples in the laboratory was also observed with bacteria in samples from two mountain streams and the river studied above collected on five occasions from July to November 1980. Table 3 shows the results of one of these experiments, in which streptomycin resistance was significantly higher ($P < 0.01$) among the isolates surviving chlorination and the frequencies of sulfanilamide resistance and tetracycline resistance decreased upon chlorination of the water. The increase in the frequency of streptomycin resistance was also noted in three of four additional laboratory chlorination experiments that are not shown; in two of these analyses, the level of confidence was $P < 0.01$, and in a third, $P = 0.04$. In the fourth experiment, the frequency of streptomycin resistance was also higher among bacteria from the chlorinated water, but the level of confidence was $P = 0.11$. We also noted other significant changes in the frequencies of antibiotic resistance phenotypes in these four experiments. In one instance, kanamycin resistance was more common among

TABLE 2. Percentages of bacterial taxonomic groups present in river and treatment plant water samples

Date (mo/day/yr)	Water used as sample source	% Isolates in following taxonomic group[a]:							
		Gram-negative rods							
		Weakly or nonfermentative						Fermentative	
		Moraxella-like *Flavobacterium*	*Pseudomonas-Alcaligenes*	*Micrococcus*	*Moraxella*	*Spirillum*	*Acinetobacter*	*Aeromonas*	Enteric[b]
3/23/81	River	35.7	16.3	20.4	0	2.0	0	0	1.0
	Flash mix treated	3.2	9.5	0	0	0	0	0	0
5/11/81	River	23.3	6.9	43.6	0	5.9	0	2.0	4.0
	Flash mix treated	0.5	7.9	2.1	0.5	0	0	1.1	1.6
	Clear well	11.9	35.7	0	0	0	0	0	7.2
6/8/81	River	47.7	11.2	19.2	0.9	0.9	1.4	2.8	3.8
	Clear well	13.0	35.9	0	0	1.1	0	0	1.1

bacteria from chlorinated water ($P < 0.01$). The proportion of sulfanilamide-resistant bacteria was lowered by chlorination in three ($P < 0.01$ in two cases and $P = 0.05$ in the third case) of the four experiments. In one experiment, tetracycline resistance was less common ($P < 0.01$) among bacteria surviving chlorination.

DISCUSSION

It is apparent that a set of highly dynamic selective processes exist within the aquatic environment of a water treatment facility. One of the primary functions of the treatment process is a selective one: the selection against bacteria as reflected in the marked decrease in bacterial numbers associated with disinfection, flocculation, and filtration of the water. In light of its overall benefit to public health, the purification of water is unquestionably necessary. However, from studies done in our laboratory, we have revealed another consequence of water treatment that could be of potential concern to public health: the selection for and survival of antibiotic-resistant SPC bacteria during water treatment.

In an earlier paper (3), we reported significantly greater frequencies of MAR SPC bacteria among isolates from drinking water samples (67.8%) as compared with the proportion of these organisms within raw-water populations (18.6%). In that study, it was not possible to distinguish whether this selection for antibiotic-

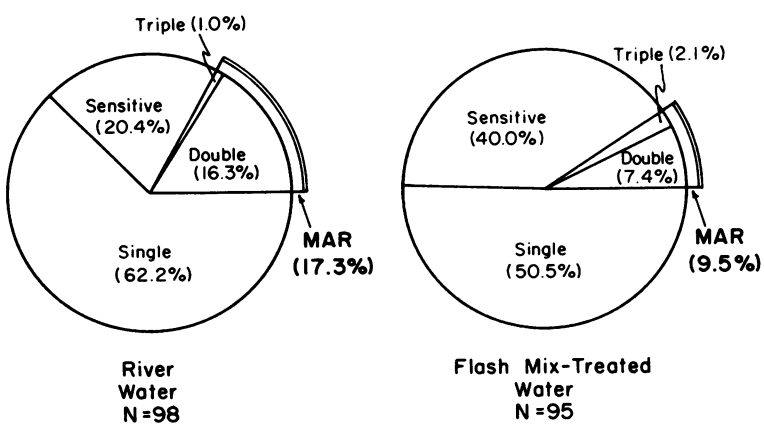

FIG. 2. Frequencies of sensitive, singly resistant, and MAR bacteria in all taxonomic groups from river and flash mix-treated waters sampled from the treatment plant on 23 March 1981. N, Number of isolates which survived purification, identification, and antibiotic resistance testing.

TABLE 2—*Continued*

% Isolates in following taxonomic group[a]:							
Unidentified, gram-negative cocci	Gram-positive cocci		Gram-positive rods			Miscellaneous unidentified	Total no. of isolates purified, identified, and tested for antibiotic resistance
	Staphylococcus	*Micrococcus*	*Bacillus*	Nonsporulating unidentified	*Arthrobacter, Corynebacterium, Streptomyces, Actinomyces*		
0	6.1	1.0	5.1	10.2	2.0	0	98
0	5.3	1.1	30.5	49.5	0	1.1	95
0	4.5	0.5	2.5	5.4	0	1.5	202
1.1	4.2	1.6	37.0	41.3	1.0	1.5	189
4.8	6.0	0	15.5	19.0	0	0	84
0	0	0	7.5	4.7	0	0	214
0	2.2	3.3	32.6	7.6	2.2	1.1	92

[a] Calculated by dividing the number of isolates within each taxonomic category by the total number of isolates identified.

[b] Enteric group includes *Enterobacter*, Centers for Disease Control group V, *Klebsiella, Serratia, Citrobacter*, and *Yersinia*.

resistant organisms was due to factors within the water treatment plant or within the pipelines of the distribution system or a combination of both. From the research reported here, we concluded that water treatment indeed can contribute to this selective phenomenon.

The selection for antibiotic-resistant bacteria has been documented in a variety of ways. One way was to consider the overall percentage of MAR bacteria in treated clear well water versus that in untreated river water. As noted in our results, river water contained microbial populations that were 15.8 and 18.2% MAR on dates when treated clear well water populations were 43.5 and 57.1% MAR, respectively. We also observed enrichment of resistance to specific antibiotics such as carbenicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline.

We found that gram-negative, antibiotic-resistant bacteria can be selected for by flash mix treatment of raw water. This selection can explain in part the increased frequencies of drug-resistant bacteria in finished drinking water. Since significant increases in the proportion of antibiotic-resistant bacteria in a river water sample can also be brought about by chlorination under controlled laboratory conditions, we also concluded that the disinfection event itself has a major impact on the selection of drug-resistant bacteria. Why certain types of resistance pheno-

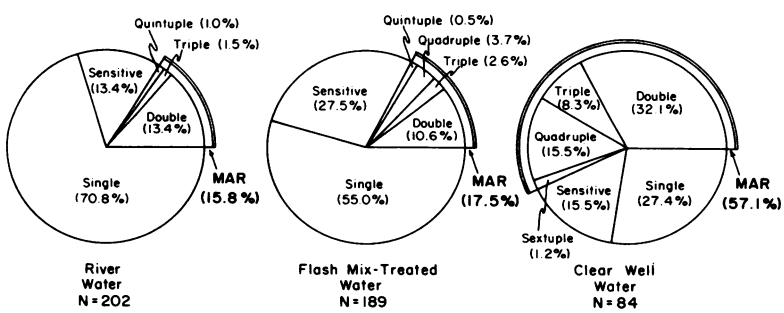

FIG. 3. Frequencies of sensitive, singly resistant, and MAR bacteria in all taxonomic groups from river, flash mix-treated, and clear well waters sampled from the treatment plant on 11 May 1981. N, Number of isolates which survived purification, identification, and antibiotic resistance testing.

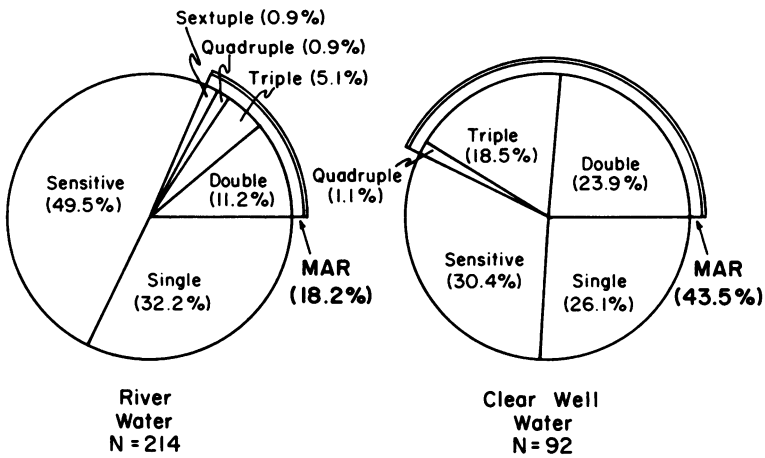

FIG. 4. Frequencies of sensitive, singly resistant, and MAR bacteria in all taxonomic groups from river and clear well waters sampled from the treatment plant on 8 June 1981. N, Number of isolates which survived purification, identification, and antibiotic resistance testing.

types, such as streptomycin resistance, are especially common among bacteria surviving chlorination is not known.

Variations were observed in the percentage of MAR organisms recovered and the resistance markers selected for after water treatment processes. It is likely that tremendous fluctuations occur within the river water populations as related to season, temperature, turbidity, total organic carbon, and chemical content (9, 12), and this probably accounts for much of the variability we have observed in regard to the types of drug-resistant SPC bacteria selected for during water treatment. Also, it is uncertain how much the resident bacteria living in the biomass coatings of the tank surfaces in the treatment plant contribute to the microbial populations of free-flowing water within the facility. Bacterial populations living in the carbon-sand filter of the facility could also be a source of antibiotic-resistant bacteria. Furthermore, much discussion has centered on the potential role of resident populations on the inner surfaces of the distribution pipes as a source of SPC organisms in distribution outlets (1, 16). Changes in all of these resident populations could have dramatic effects on the types of bacteria within the finished drinking water.

Why MAR bacteria survive the environment within the waters of the treatment facility is not yet understood. Perhaps they are lodged within particulates which protect them from the chlo-

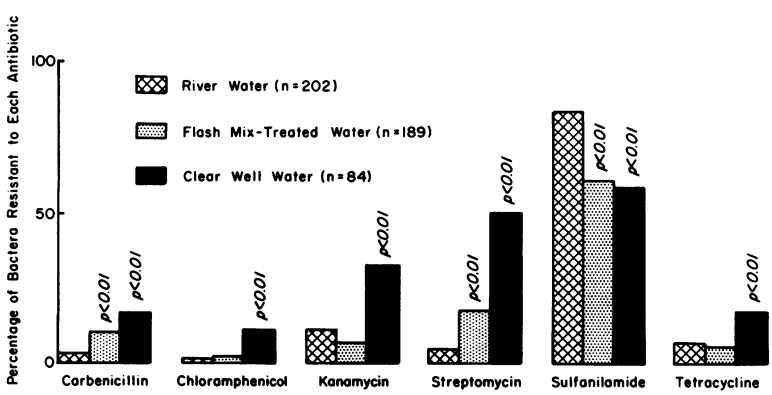

FIG. 5. Frequencies of isolates resistant to each of the test antibiotics. River and treatment plant waters were collected on 11 May 1981. N, Number of isolates which survived purification, identification, and antibiotic resistance testing; P values are indicated only where $P \leq 0.05$ in comparisons of flash mix-treated or clear well isolates relative to isolates in the river water.

TABLE 3. Frequencies of antibiotic-resistant bacteria from laboratory-chlorinated surface water

Phenotype	Occurrence				P[c]
	Before chlorination[a]		After chlorination[b]		
	No.	% All isolates analyzed	No.	% All isolates analyzed	
Streptomycin resistant	68	17.3	53	31.7	0.01
Kanamycin resistant	25	6.4	16	9.6	0.18
Chloramphenicol resistant	10	2.5	1	0.6	0.13
Tetracycline resistant	26	6.6	3	1.8	0.02
Sulfanilamide resistant	311	79.1	95	56.9	0.01
Sensitive to all five antibiotics	71	18.1	38	22.8	0.20
MAR	94	23.9	33	20.0	0.28

[a] SPC, 96 CFU/ml; total number of isolates analyzed, 393.
[b] SPC, 0.44 CFU/ml; total number of isolates analyzed, 167. Calcium hypochlorite was added at 1.5 mg/liter of free residual chlorine for 60 min.
[c] Significance of the difference between the percentages of isolates analyzed before and after chlorination.

rine and other chemicals (9). They may also be chlorine tolerant (4, 14, 15, 17), with the free residual chlorine in the water acting as a selective agent to increase their relative numbers in the population. Capsule formation has been linked to the ability of bacteria to survive in chlorinated distribution water (15). Such a layer might protect the isolates against antibiotics or any potentially bactericidal substances in the water treatment facility. Other studies under way in our laboratory indicate that MAR bacteria surviving water treatment are more typically tolerant to metal salts such as $CuCl_2$, $Pb(NO_3)_2$, and $ZnCl_2$ (J. J. Calomiris, J. L. Armstrong, D. S. Shigeno, and R. J. Seidler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, Q127, p. 221). MAR bacteria from the inner surfaces of distribution pipes are also more typically tolerant to metal salts (J. J. Calomiris, J. L. Armstrong, and D. S. Shigeno, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, Q107, p. 227). This may reflect a coincident selection of the MAR phenotype among bacteria that are able to live in the high concentrations of metals in the biofilms coating the inner pipe surfaces.

Others have commented on the potential hazard that drug-resistant organisms pose to public health, and caution might be advisable in cases where antibiotic-resistant opportunistic pathogens (19) are present in drinking water that is consumed by patients using immunosuppressive drugs or undergoing chemotherapy. In some water supply systems, SPCs exceed 1,000 CFU/ml (5, 11). Patients who are prone to infections by opportunistic pathogens might be advised in such cases to consume boiled water. Also, as we mentioned in a previous publication (3), we raise the issue that water treatment may be a contributing factor to the selection of antibiotic-resistant SPC bacteria in the environment.

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