APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1982, p. 371–377
0099-2240/82/020371-07$02.00/0

Vol. 43, No. 2

Effect of UV Light Disinfection on Antibiotic-Resistant Coliforms in Wastewater Effluents

MARK C. MECKES†

Wastewater Research Division, Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268

Received 15 June 1981/Accepted 13 October 1981

Total coliforms and total coliforms resistant to streptomycin, tetracycline, or chloramphenicol were isolated from filtered activated sludge effluents before and after UV light irradiation. Although the UV irradiation effectively disinfected the wastewater effluent, the percentage of the total surviving coliform population resistant to tetracycline or chloramphenicol was significantly higher than the percentage of the total coliform population resistant to those antibiotics before UV irradiation. This finding was attributed to the mechanism of R-factor-mediated resistance to tetracycline. No significant difference was noted for the percentage of the surviving total coliform population resistant to streptomycin before or after UV irradiation. Multiple drug resistance patterns of 300 total coliform isolates revealed that 82% were resistant to two or more antibiotics. Furthermore, 46% of these isolates were capable of transferring antibiotic resistance to a sensitive strain of *Escherichia coli*.

In 1959, Watanabe (31) discovered that some *Escherichia coli* strains could transfer antibiotic resistance to antibiotic-sensitive strains of *Shigella* spp. Subsequent research has demonstrated that bacteria carrying transmissible R-factors are responsible for the spread of multiple antibiotic resistance among members of the *Enterobacteriaceae* (such as *E. coli, Salmonella typhi*, and *Shigella dysenteriae*) *Aeromonas* and *Yersinia* species (4), *Pseudomonas aeruginosa* (21), and *Vibrio cholerae* (34).

Transmission of R-factors in the *Enterobacteriaceae* usually occurs by conjugation, which involves a specialized structure called the "sex pilus" and requires cell-to-cell contact or cell-pilus-cell contact. The ability and the efficiency of different bacterial strains to donate or receive R-factors varies (8). Transmission of R-factors by conjugation is rapid and may spread rapidly among bacteria (31).

When bacteria which carry transmissible R-factors (R^+ bacteria) are ingested by a human host, the R-factors may transfer into commonly occurring bacteria of the gastrointestinal tract (32). These organisms may subsequently transfer this resistance to pathogenic organisms, resulting in reduced efficacy of antimicrobial chemotherapy in the event of an infection. In vivo studies have shown that when individuals carrying R^+ bacteria are subjected to antibiotic therapy, these organisms flourish and transfer their resistance to other bacteria (25).

From late 1968 to early 1981, Central America was afflicted by an R^+ *S. dysenteriae* pandemic (11). During the first year of the epidemic, in Guatemala alone, 12,500 deaths were recorded. The causative organism was spread mainly by contaminated water and carried resistance to streptomycin, tetracycline, chloramphenicol, and sulfadiazine. Other outbreaks involving R^+ pathogens have been reported elsewhere (1, 24).

Several researchers have pointed out that wastewater, treated or untreated, is a primary contributor of bacteria to the aquatic ecosystem (12, 16, 17, 20, 27, 29). Studies have been conducted which demonstrate that significant numbers of multiple drug-resistant coliforms occur in rivers (17), bays (9), bathing beaches (28), and coastal canals (13). Waters contaminated by bacteria capable of transferring drug resistance are of great concern since there is the potential for transfer of antibiotic resistance to a pathogenic species.

Available information shows that conventional wastewater purification methods without disinfection are not adequate for removal of antibiotic-resistant bacteria (14, 15, 29). Wastewater disinfection is, therefore, the only means whereby communities can limit the number of antibiotic-resistant bacteria in the water environment since it seems unlikely that antibiotic chemotherapy will be reduced.

Historically, chlorination has been used in the United States for wastewater disinfection (33).

† Present address: Defense Property Disposal Service, Battle Creek, MI 49016.

Downloaded from http://aem.asm.org/ on December 27, 2016 by University of North Texas Libraries

However, chlorine residuals have been shown to be toxic to aquatic wildlife (3). An alternative method of disinfection is to use UV light irradiation at 253.7 nm. UV disinfection does not produce a toxic residual and is an efficient bactericide (23). Studies have been conducted which indicate that UV light can be used effectively for disinfection of municipal secondary wastewater effluents (18, 26). One of these studies has projected that UV disinfection of activated sludge effluents may be the most cost effective alternative to chlorination (26).

Several communities in the United States have selected UV light for disinfection of their effluent wastewater. Therefore, the effect of UV light disinfection on antibiotic-resistant coliforms in municipal wastewater effluents should be ascertained to determine whether the use of this disinfection technique will significantly reduce the ratio of antibiotic-resistant bacteria to antibiotic-sensitive bacteria entering receiving waters from wastewater treatment facilities. This study was designed to determine the effects of UV light disinfection on antibiotic-resistant total coliforms in municipal effluents.

MATERIALS AND METHODS

UV irradiation of filtered activated sludge effluents. On the day of each experiment, clarified activated sludge effluent was collected ahead of the disinfection stage at the Fairfield Wastewater Treatment Plant operated by the City of Fairfield, Ohio. This plant (6,000,000 gallons per day) uses conventional activated sludge after primary clarification for treatment of domestic wastewater.

After collection, the effluent was trucked to the U.S. Environmental Protection Agency R. A. Taft Laboratory Pilot Plant, where the effluent was pumped through a mixed media pressure filter, a Brooks Sho-Rate roto-meter, and a UV sterilizer (Aquafine model DP-10-2U, Burbank, Calif.). The filtration system was used to limit the suspended solids of the secondary effluent. This was necessary to insure the rapid filtration of up to a 500-ml sample through membrane filters for enumeration of antibiotic-resistant total coliforms.

The Aquafine UV unit was designed to provide a ≥99% bactericidal reduction across the unit at maximum rated flow. The unit used for this study had a maximum rated flow capacity of 1.5 gallons (5.68 liters) per min (gpm) on freshwater. The UV dose at this flow rate was 45,000 $\mu Ws/cm^2$ as determined by the manufacturer. Since the DP-10-2U sterilizer was designed for sterilization of freshwater, the efficiency of the unit for disinfection of wastewaters was expected to be somewhat less. The DP-10-2U utilized a quartz U-shaped tube of 160 ml (total volume) through which the treated effluent flowed. Two germicidal 8-W lamps (1.5 W total UV output at 254 nm) were positioned on opposing sides of the U-shaped tube such that the greatest distance the light had to travel to penetrate the liquid traversing the tube was 2 in. A polished stainless steel housing surrounded the U-shaped tube and lamp assemblies to provide a reflective surface for better utilization of the available UV light.

The dosage of UV light used in these experiments was chosen to provide disinfection as defined by achieving <1,000 total coliforms per 100 ml and not to deliberately induce other changes in the bacterial population.

Sample collection. The above system was operated for approximately 30 min before sample collection to assure that all liquid-carrying lines were receiving fresh effluent and to allow sufficient warm-up time for the UV lamps. Just before sample collection, the sample line was opened fully and flushed for approximately 1 min. The flow rate was adjusted to compensate for the resulting pressure drop. A Tygon U-shaped tube was installed at the discharge to provide additional residence time so that all samples were temporally related. All samples were collected in sterile glass reagent bottles.

Isolation and enumeration of bacteria. Samples before and after UV treatment were anlayzed for total coliform densities by membrane filter techniques as outlined in *Microbiological Methods for Monitoring the Environment* (30). Antibiotic-resistant total coliforms were enumerated by use of the same techniques. However, the *m*-Endo medium (Difco Laboratories) used for these isolations contained one of the three following selection antibiotics at 20-μg/ml concentration: streptomycin, tetracycline, or chloramphenicol. Fifty colonies from each selection antibiotic class before UV treatment and fifty colonies from each selection antibiotic class after UV treatment were picked at random from the membrane filter plates used for enumeration after 5 of the 12 enumeration experiments. These colonies were purified by streaking them onto eosin methylene blue agar (Difco) containing a 20-μg/ml concentration of the original selection antibiotic and incubated at 35°C overnight. A single, well-isolated colony from the eosin methylene blue plate was then inoculated to a nutrient agar (Difco) slant which, after overnight incubation at 35°C, served as a stock culture.

Multiple drug resistance testing. Drug resistance patterns of the above cultures were determined by the single disk diffusion method of Bauer et al. (2), except that nutrient broth (Difco) served as the primary growth medium, and incubation was at 35°C. The following antibiotic disks were used to determine patterns of resistance (concentrations in micrograms per disk; all from Difco): ampicillin (10), cephalothin (30), chloramphenicol (10), colisten (10), gentamicin (10), kanamycin (30), streptomycin (10), and tetracycline (10). Strains with intermediate resistance to an antibiotic were classified as resistant to that antibiotic.

Transfer of drug resistance studies. Antibiotic-resistant isolates before and after UV treatment were utilized as prospective donors of resistance to a plasmidless, completely antibiotic-sensitive strain of *E. coli*, designated *E. coli* K-12 C600 (F^- Azi^r) and provided by John M. Trela, University of Cincinnati. This recipient strain is phenotypically Lac^-, yielding clear colonies on MacConkey agar plates, and is resistant to a 100-μg/ml concentration of sodium azide. Transfer procedures were carried out by mixing 0.1 and 0.2 ml of overnight broth cultures of the prospective donor and recipient, respectively, in 2 ml of sterile

Downloaded from http://aem.asm.org/ on December 27, 2016 by University of North Texas Libraries

TABLE 1. Total coliforms and antibiotic-resistant coliforms from effluents before and after UV irradiation

Date (1979)	Log_{10} total coliforms/100 ml							
	Before UV irradiation				After UV irradiation			
	NA	Sm^r	Te^r	Cm^r	NA	Sm^r	Te^r	Cm^r
3/15	6.38	6.15	4.83	3.67	2.93	2.54	2.30	0.97
3/16	6.56	6.34	5.04	3.92	2.63	2.28	2.00	0.72
3/27	<5.60[b]	<4.90	<3.90	<2.60	<2.30	<1.60	<1.30	<0.30
3/28	6.28	5.92	4.71	3.77	3.69	3.38	2.82	2.00
3/29	6.28	5.88	4.69	3.77	2.38	2.00	1.40	0.58
4/4	6.20	5.78	4.72	3.74	<2.30	<1.60	<1.30	<0.30
4/5	5.59	5.32	4.30	2.74	2.32	2.00	1.40	0.11
4/10	6.30	5.97	4.77	3.59	2.73	2.40	2.20	0.99
4/11	6.11	5.72	4.52	3.36	2.56	2.20	1.64	0.67
4/12	6.40	5.96	4.84	3.57	3.56	2.93	2.45	1.34
4/17	5.95	5.58	4.50	3.15	2.32	1.94	1.60	0.43
4/18	6.11	5.82	4.54	3.45	2.83	2.53	2.00	1.00
Mean[b]	6.20	5.86	4.68	3.52	2.80	2.42	1.98	0.88

[a] NA, No antibiotic; Sm, streptomycin; Te, tetracycline; Cm, chloramphenicol. All antibiotics were at 20 μg/ml.

[b] Numbers preceded by < indicate too few colonies to provide a reliable number and therefore were not used to calculate means.

broth and incubating at 35°C overnight. A heavy loopful of each of these mating mixtures was smeared onto MacConkey agar (BBL Microbiology Systems) plates containing 100 μg of sodium azide per ml and 20 μg of the selection antibiotic per ml used to isolate the prospective donor strain. The media used were selective for antibiotic-resistant recombinants of the *E. coli* K-12 strain since growth of the prospective donor was prevented by sodium azide, and growth of the prospective recipient was prevented by an antibiotic. After incubation at 35°C for 24 h, a single, well-isolated lactose-negative colony was placed in nutrient broth and tested for antibiotic resistance patterns by the above mentioned method to ascertain whether all or part of the resistance pattern was transferred from the donor strain to the recipient strain. Controls were run with donor and recipient strains to assure the validity of the transfer experiments.

RESULTS

Enumeration of antibiotic-resistant total coliforms. Activated sludge effluents were irradiated at a flow rate of 1.5 gpm, which resulted in a total coliform density below 1,000 total coliforms per 100 ml (Table 1) on all but two of the samples. Similarly, two of the samples after UV treatment resulted in too few coliforms to be detected. The data in Table 1 also show that UV irradiation effectively reduced the number of antibiotic-resistant total coliforms in the activated sludge effluents.

Table 2 shows the percentage of antibiotic-resistant coliforms before and after UV disinfection. Means from Table 2 show that UV irradiation of the effluents resulted in a decrease in the percentage of surviving total coliforms resistant to streptomycin and an increase in the percentage of surviving total coliforms resistant to tetracycline or chloramphenicol.

Analysis-of-variance techniques were used to determine the significance of the change in percentage of antibiotic resistance observed in the surviving total coliform population after UV irradiation. The data from the 2 days which

TABLE 2. Percentage of antibiotic-resistant total coliforms from effluents before and after UV irradiation

Date (1979)	% antibiotic-resistant total coliforms[a]					
	Before UV irradiation			After UV irradiation		
	Sm^r	Te^r	Cm^r	Sm^r	Te^r	Cm^r
3/15	57.0	2.8	0.2	41.4	23.8	1.1
3/16	60.9	3.0	0.2	43.0	23.6	1.2
3/27	Indeterminant			Indeterminant		
3/28	43.1	2.7	0.3	49.9	13.5	2.0
3/29	40.2	2.6	0.3	42.4	10.4	1.6
4/4	37.0	3.3	0.3	Indeterminant		
4/5	53.8	5.1	0.1	47.0	11.6	0.6
4/10	47.8	3.0	0.2	46.3	28.7	1.8
4/11	40.9	2.6	0.2	44.4	12.2	1.3
4/12	37.0	2.8	0.2	23.3	7.8	0.6
4/17	42.4	3.6	0.2	41.6	18.9	1.3
4/18	50.4	2.7	0.2	50.8	15.2	1.6
Mean	46.4	3.1	0.2	43.0	16.6	1.3

[a] Calculated by dividing the number of total coliforms resistant to a specific antibiotic by the number of total coliforms in the same sample and multiplying by 100. Sm, streptomycin; Te, tetracycline; Cm, chloramphenicol. All antibiotics were at 20 μg/ml.

Downloaded from http://aem.asm.org/ on December 27, 2016 by University of North Texas Libraries

TABLE 3. Analysis of variance on the percentage of antibiotic-resistant total coliforms before and after UV irradiation

Resistance to:	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Streptomycin	Treatment	1	94.178	94.178	1.54
	Error	18	1100.514	61.140	
	Total	19	1194.692		
Tetracycline	Treatment	1	908.552	908.552	38.07[a]
	Error	18	429.61	23.867	
	Total	19	1338.162		
Chloramphenicol	Treatment	1	6.050	6.05	55.00[a]
	Error	18	1.978	0.11	
	Total	19	8.028		

[a] Significant at the 0.01 level.

resulted in too few colonies to yield a reliable number were deleted from the analysis. Table 3 shows the results from these tests. The percentages of tetracycline-resistant and chloramphenicol-resistant total coliforms increased significantly after UV irradiation, while there was no significant difference between the percentage of streptomycin-resistant total coliforms present in the effluent before and after UV treatment.

Multiple antibiotic-resistant total coliforms. A total of 300 colonies were picked at random from the *m*-Endo plates containing streptomycin, tetracycline, or chloramphenicol. One-half of these isolates were from effluents sampled before UV irradiation; the rest were from effluents sampled after UV irradiation. Equal numbers of colonies were picked to represent each selection antibiotic used. These isolates were tested for resistance to eight different antibiotics. The most common antibiotic resistance patterns observed for the total coliform isolates are presented in Tables 4, 5, and 6.

Of the 300 isolates examined, 55 were resistant to only one of the antibiotics tested. Of these 55 isolates, 25 were resistant to streptomycin, 27 were resistant to tetracycline, and only 3 were resistant to chloramphenicol. These data clearly indicate that the majority of antibiotic-resistant coliforms from these effluents were resistant to two or more drugs. None of the strains tested showed resistance to colisten, and only one strain was resistant to gentamicin.

UV light treatment of the effluent appears to have some effect on the multiple antibiotic resistance patterns observed in total coliform isolates. From effluents before UV treatment of 50 strains isolated on media containing streptomycin, 18 exhibited resistance to three or more antibiotics (Table 4). However, of the 50 strains isolated in the same manner from UV-treated effluents, 24 exhibited resistance to three or more antibiotics. Similarly, the number of strains exhibiting resistance to three or more antibiotics isolated on media containing tetracycline (Table 5) were 17 and 30, respectively. This apparent selection by UV treatment for multiple antibiotic resistance in total coliform strains was not observed in strains isolated on media containing chloramphenicol (Table 6) because most of these isolates (89/100) exhibited resistance to three or more antibiotics, regardless of irradiation.

For certain antibiotic combinations, selection of antibiotic-resistant total coliforms by UV treatment is shown in Table 6 for strains isolated on media containing chloramphenicol. Before UV treatment, the strains exhibiting the Sm Te Cm Am and the Sm Te Cm Km Am resistance

TABLE 4. Antibiotic resistance patterns encountered in total coliforms isolated on media containing streptomycin

Resistance pattern[a]	No. of isolates[b] (%) from samples	
	Before UV irradiation	After UV irradiation
Sm	11 (22)	14 (28)
Sm Te	16 (32)	6 (12)
Sm Km	2 (4)	1 (2)
Sm Am	3 (6)	4 (8)
Sm Cr	0	1 (2)
Sm Te Cm	0	4 (8)
Sm Te Km	1 (2)	1 (2)
Sm Te Am	8 (16)	7 (14)
Sm Cm Am	1 (2)	0
Sm Te Cm Km	2 (4)	1 (2)
Sm Te Cm Am	0	3 (6)
Sm Te Km Am	3 (6)	6 (12)
Sm Te Cm Km Am	3 (6)	2 (4)

[a] Sm, streptomycin; Km, kanamycin; Te, tetracycline; Cm, chloramphenicol; Am, ampicillin; Cr, cephalothin.

[b] Total of 50. None of these isolates displayed resistance to colisten or gentamicin.

Downloaded from http://aem.asm.org/ on December 27, 2016 by University of North Texas Libraries

TABLE 5. Antibiotic resistance patterns encountered in total coliforms isolated on media containing tetracycline

Resistance pattern[a]	No. of isolates[b] (%) from samples	
	Before UV irradiation	After UV irradiation
Te	17 (34)	10 (20)
Sm Te	13 (26)	8 (16)
Te Am	3 (6)	2 (4)
Sm Te Cm	0	4 (8)
Sm Te Km	1 (2)	1 (2)
Sm Te Am	9 (18)	12 (24)
Sm Te Cm Am	2 (4)	2 (4)
Sm Te Km Am	5 (10)	7 (14)
Sm Te Cr Am	0	1 (2)
Sm Te Cm Km Am	0	3 (6)

[a] Sm, streptomycin; Km, kanamycin; Te, tetracycline; Cm, chloramphenicol; Am, ampicillin; Cr, cephalothin.

[b] Total of 50. None of these isolates displayed resistance to colisten or gentamicin.

patterns made up a total of 40% of all isolates. After UV treatment, 70% of all isolates from media containing chloramphenicol exhibited one or the other of these resistance patterns. These resistance patterns were observed less frequently in strains isolated on media containing streptomycin or tetracycline. However, the Sm Te Cm Am and the Sm Te Cm Km Am resistance patterns occurred more frequently (10 occurrences) in strains taken from UV-treated effluents and isolated on media containing streptomycin or tetracycline (Tables 4 and 5) than in strains taken from effluents before UV treatment and isolated in the same manner (5 occurrences).

Transfer of antibiotic resistance. The 300 strains were tested for their ability to transfer resistance to an antibiotic-sensitive strain of *E. coli* K-12. The method used required that resistance to the selection antibiotic, upon which the donor strain was isolated, be transferred to the recipient strain. Therefore, it is possible that transfer of resistance to antibiotics other than the antibiotic used for selection may have occurred without being detected. All recombinant strains were examined for multiple antibiotic resistance patterns as described above.

The percentage of coliforms transferring resistance to the antibiotic-sensitive strain varied (Table 7). This variation was dependent upon the selection antibiotic used and UV irradiation treatment. Overall, 138 of the 300 isolates (46%) transferred antibiotic resistance to the antibiotic-sensitive strain. Over 86% of the recombinants exhibited resistance to all of the antibiotics to which the donor strains were resistant. This high efficiency was probably the result of allowing mixed culture growth of donor and recipient strains to proceed for 16 h.

It is interesting to note that although there was no significant increase observed in the percentage of streptomycin-resistant total coliforms surviving UV irradiation, the ability of this population to transfer antibiotic resistance increased. This increase in ability to transfer drug resistance was not observed for coliforms isolated from media containing tetracycline or chloramphenicol.

Coliforms isolated on media containing chloramphenicol showed somewhat less ability to transfer resistance after UV irradiation. However, this reduction in ability to transfer is probably not significant. Transfer of resistance to six antibiotics was noted in two of the total coliform strains taken from effluents before UV treatment and isolated on media containing chloramphenicol.

DISCUSSION

Ampicillin, streptomycin, and tetracycline are probably the most commonly used antibiotics in human medicine. Therefore, it is reasonable to assume that a higher frequency of occurrence of bacteria resistant to these antibiotics would be expected in wastewater effluents. It is important

TABLE 6. Antibiotic resistance patterns encountered in total coliforms isolated on media containing chloramphenicol

Resistance pattern[a]	No. of isolates[b] (%) from samples	
	Before UV irradiation	After UV irradiation
Cm	2 (4)	1 (2)
Sm Cm	1 (2)	0
Te Cm	1 (2)	1 (2)
Cm Am	0	5 (10)
Sm Te Cm	4 (8)	0
Sm Cm Am	3 (6)	0
Te Cm Km	0	1 (2)
Te Cm Cr	3 (6)	0
Te Cm Am	2 (4)	1 (2)
Sm Te Cm Km	5 (10)	1 (2)
Sm Te Cm Cr	1 (2)	0
Sm Te Cm Am	11 (22)	18 (36)
Sm Cm Km Am	0	1 (2)
Te Cm Km Am	1 (2)	0
Te Cm Cr Am	1 (2)	0
Sm Te Cm Km Am	9 (18)	17 (34)
Sm Te Cm Cr Am	3 (6)	2 (4)
Sm Te Cm Gm Km Am	1 (2)	0
Sm Te Cm Km Cr Am	2 (4)	2 (4)

[a] Sm, streptomycin; Km, kanamycin; Te, tetracycline; Gm, gentamicin; Cm, chloramphenicol; Am, ampicillin; Cr, cephalothin.

[b] Total of 50. None of these isolates displayed resistance to colisten.

Downloaded from http://aem.asm.org/ on December 27, 2016 by University of North Texas Libraries

TABLE 7. Total coliform isolates transferring antibiotic resistance

Selection antibiotic	No. of isolates (%)[a]	
	Before UV irradiation	After UV irradiation
Streptomycin	19 (38)	32 (64)
Tetracycline	19 (38)	19 (38)
Chloramphenicol	26 (52)	23 (46)
Total	64 (43)	74 (49)

[a] Overall total, 138 (46).

to remember that isolates in these experiments were tested for resistance to only eight antibiotics, and, consequently, resistance to other antibiotics may be carried by these organisms.

The mean percentage of all total coliform isolates capable of transferring all or part of their antibiotic resistance (46%) was identical to that observed by Fontaine and Hoadley (10) for drug-resistant fecal coliforms isolated from undisinfected municipal wastewaters. Similarly, Sturtevant and Feary (29) reported that 43% of the drug-resistant total coliforms, isolated from undisinfected municipal wastewaters (before and after biological trickling-filter treatment), were capable of transferring resistance to a sensitive strain of *E. coli*. These same researchers observed the same lack of resistance to colisten and gentamicin in total coliform isolates that was observed in this study.

The increase in the percentage of total coliforms resistant to tetracycline or chloramphenicol after exposures to UV light may be explained by the presence of an additional R-factor which mediates UV resistance. An R-factor mediating UV resistance has been characterized in *E. coli* K-12 by Marsh and Smith (22) and noted in *S. typhimuriun* by Drabble and Stocker (7). Association of a UV R-factor with resistance to specific antibiotics could also explain the increased occurrence of the Sm Te Cm Am and Sm Te Cm Km Am resistance patterns observed in isolates from UV-treated effluents. However, no association between R-factors which mediate UV resistance and R-factors which mediate resistance to specific antibiotics has been found.

Alternatively, the increase in the percentage of total coliforms resistant to tetracycline or chloramphenicol after UV irradiation may be explained by the mechanism of R-factor-mediated resistance to tetracycline. R-factor-mediated resistance to aminoglycoside antibiotics such as streptomycin, kanamycin, and gentamicin has been found to be associated with specific enzymes which modify or hydrolyze the antibiotic to a more innocuous form (8). Similarly, production of chloramphenicol acetyltransferase, which enzymatically inactivates chloramphenicol, is coded for by R-factors (6, 8). However, R-factor-mediated resistance to tetracycline is not associated with enzymatic modification of tetracycline. The resistance is due to accumulation within the cell envelope of specific proteins which inhibit transport of tetracycline to target ribosomes of the cell (5).

The specific protein responsible for bacterial resistance to tetracycline (tet protein) may absorb sufficient UV light at 254 nm to afford these bacteria some degree of protection from UV irradiation. This could explain why an increase in the percentage of surviving tetracycline-resistant total coliforms was noted after UV treatment. The accompanying increase in surviving chloramphenicol-resistant total coliforms was not due to chloramphenicol resistance, but to concomitant resistance to tetracycline. This becomes apparent when the percentage of strains isolated on media containing chloramphenicol and resistant to tetracycline, both before and after UV treatment, is compared. No significant difference in the percentage of these strains exhibiting both chloramphenicol and tetracycline resistance was noted (88% before UV versus 86% after UV). Since a high percentage of chloramphenicol-resistant coliforms in effluents sampled before UV treatment was concomitantly resistant to tetracycline, the percent increase in surviving chloramphenicol-resistant coliforms after UV treatment cannot be attributed to chloramphenicol resistance alone because there was no decrease in the number of isolates concomitantly resistant to tetracycline. Further work is necessary to confirm this notion.

It is evident from this work as well as from the work of others (10, 13–15, 29) that antibiotic-resistant coliforms are entering the aquatic environment via treated municipal wastewater effluents. This work demonstrates that UV light disinfection can effectively reduce the number of total coliforms both sensitive and resistant to antibiotics in an activated sludge effluent. This work also points out that there is a significant increase in the percentage of the surviving total coliform population resistant to tetracycline and chloramphenicol after UV irradiation.

This study concerned itself with UV disinfection. There is little information available which discusses the effect of other disinfectants on antibiotic-resistant organisms. Additional investigations should be conducted to determine what effect other wastewater disinfectants, such as chlorine or ozone, may have on the antibiotic-resistant fraction of the bacterial population. There is an additional need to determine the sanitary significance of the results of such investigations.

ACKNOWLEDGMENTS

I thank John Trela of the Biological Science Department,

Downloaded from http://aem.asm.org/ on December 27, 2016 by University of North Texas Libraries

University of Cincinnati, for providing the recipient *E. coli* strain used in this study and invaluable guidance. I also thank P. V. Scarpino of the University of Cincinnati Civil and Environmental Engineering Department for valuable discussions and helpful criticism.

LITERATURE CITED

1. **Anonymous.** 1973. Current trends. Follow-up on chloramphenicol-resistant *Salmonella typhi*—Mexico. Morbid. Mortal. Weekly Rep. **22**:159.
2. **Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**:493–496.
3. **Brungs, W. A.** 1973. Effects of residual chlorine on aquatic life. J. Water Pollut. Ctl. Fed. **45**:2180–2193.
4. **Chatterjee, A. K., and M. P. Starr.** 1972. Transfer among *Erwinia* spp. and other enterobacteria of antibiotic resistance carried on R-factors. J. Bacteriol. **112**:576–584.
5. **Chopra, I., and T. G. B. Howe.** 1978. Bacterial resistance to tetracyclines. Microbiol. Rev. **42**:707–724.
6. **Davies, J., and D. I. Smith.** 1978. Plasmid-determined resistance to antimicrobial agents. Annu. Rev. Microbiol. **32**:496–518.
7. **Drabble, W. T., and B. A. D. Stocker.** 1968. R. (transmissable drug resistance) factors in *Salmonella typhimuriun*: pattern of transduction by phage P22 and ultraviolet protection effect. J. Gen. Microbiol. **53**:109–123.
8. **Falkow, S.** 1975. Infectious multiple drug resistance. Pion Limited, London.
9. **Feary, T. W., A. B. Sturtevant, Jr., and J. Lankford.** 1972. Antibiotic-resistant coliforms in fresh and salt water. Arch. Environ. Health **25**:215–220.
10. **Fontaine, T. D., and A. W. Hoadley.** 1976. Transferable drug resistance associated with coliforms isolated from hospital and domestic sewage. Health Lab. Sci. **13**:238–245.
11. **Gangarosa, E. J., J. V. Bennett, C. Wyatt, P. E. Pierce, J. Olarte, P. M. Hernandes, V. Vazquez, and M. D. Bessudo.** 1972. An epidemic-associated episome? J. Infect. Dis. **126**:215–218.
12. **Geldreich, E. E.** 1972. Water-borne pathogens, p. 207–241. *In* R. Mitchell (ed.), Water pollution microbiology. Wiley-Interscience, New York.
13. **Goyal, S. M., C. P. Gerba, and J. L. Melnick.** 1979. Transferable drug resistance in bacteria of coastal canal water and sediment. Water Res. **13**:349–356.
14. **Grabow, W. O. K., O. W. Prozesky, and J. S. Burger.** 1975. Behavior in a river and dam of coliform bacteria with transferable or non-transferable drug resistance. Water Res. **9**:777–782.
15. **Grabow, W. O. K., O. W. Prozesky, and L. S. Smith.** Drug resistance of coliform bacteria in hospital and city sewage. Antimicrob. Agents Chemother. **3**:175–180.
16. **Grabow, W. O. K., O. W. Prozesky, and L. S. Smith.** 1974. Drug-resistant coliforms call for a review of water quality standards. Water Res. **8**:1–9.
17. **Grabow, W. O. K., M. VanZyl, and O. W. Prozesky.** 1976. Behavior in conventional sewage purification processes of coliform bacteria with transferable or non-transferable drug resistance. Water Res. **10**:717–723.
18. **Johnson, J. D., K. Aldrich, D. E. Francisco, T. Wolff, and M. Elliott.** 1979. UV disinfection of secondary effluent, p. 108–116. *In* A. D. Venosa (ed.), Progress in wastewater disinfection technology. Proceedings of the National Symposium, EPA-600/9-79-018. U.S. Environmental Protection Agency, Cincinnati, Ohio.
19. **Kelch, W. J., and J. S. Lee.** 1978. Antibiotic resistance patterns of gram-negative bacteria isolated from environmental sources. Appl. Environ. Microbiol. **36**:450–456.
20. **Linton, K. B., M. H. Richmond, R. Bevau, and W. A. Gillespie.** 1974. Antibiotic resistance and R-factors in coliform bacilli isolated from hospital and domestic sewage. J. Med. Microbiol. **7**:91–103.
21. **Lowbury, E. J. L., J. R. Balb, and E. Roe.** 1972. Clearance from a hospital of gram-negative bacilli that transfer carbenicillin resistance to *Pseudomonas aeruginosa*. Lancet **ii**:941–945.
22. **Marsh, E. B., Jr., and D. H. Smith.** 1969. R-factors improving survival of *Escherichia coli* K-12 after ultraviolet irradiation. J. Bacteriol. **100**:128–139.
23. **Oliver, B. G., and E. G. Cosgrove.** 1975. The disinfection of sewage treatment plant effluents using ultraviolet light. Can. J. Chem. Eng. **53**:170–174.
24. **Paniker, C. K., and K. N. Vimala.** 1972. Transferable chloramphenicol resistance on *Salmonella typhi*. Nature (London) **239**:109–110.
25. **Pollack, M., P. Charache, R. E. Nieman, M. P. Jeit, J. A. Reinhardt, and P. H. Hardy, Jr.** 1972. Factors influencing colonization and antibiotic resistance patterns of gram-negative bacteria in hospital patients. Lancet **ii**:668–671.
26. **Scheible, O., G. Binkowski, and T. Mulligan.** 1979. Full-scale evaluation of ultraviolet disinfection of a secondary effluent. *In* A. D. Venosa (ed.), Progress in wastewater disinfection technology. Proceedings of the National Symposium EPA-600/9-79-018. U.S. Environmental Protection Agency, Cincinnati, Ohio.
27. **Smith, H. W.** 1970. Incidence in river water of *Escherichia coli* containing R-factors. Nature (London) **228**:1286–1288.
28. **Smith, H. W.** 1971. Incidence of R^+ *Escherichia coli* in coastal bathing waters of Britain. Nature (London) **234**:155–156.
29. **Sturtevant, A. B., Jr. and T. W. Feary.** 1969. Incidence of infectious drug resistance among lactose-fermenting bacteria isolated from raw and treated sewage. Appl. Microbiol. **18**:918–924.
30. **U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory.** 1978. Microbiological methods for monitoring the environment, EPA-600/8-78-017. U.S. Environmental Protection Agency, Cincinnati, Ohio.
31. **Watanabe, T.** 1962. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. **27**:87–115.
32. **Watanabe, T.** 1971. Infectious drug resistance in bacteria. Curr. Top. Microbiol. Immunol. **56**:43–98.
33. **White, G. C.** 1972. Handbook of chlorination. Van Nostrand-Reinhold, New York.
34. **Yokata, T., T. Kasuga, M. Kaneko, and S. Kuwahara.** 1972. Genetic behavior of R-factors in *Vibrio cholerae*. J. Bacteriol. **109**:440–442.

Downloaded from http://aem.asm.org/ on December 27, 2016 by University of North Texas Libraries