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Drug Resistance of Coliform Bacteria in Hospital and City Sewage

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The number and properties of drug-resistant coliform bacteria in hospital and city sewage were compared. There was little difference in the counts of organisms with nontransferable resistance to one or more of 13 commonly used drugs. An average of 26% of coliforms in hospital waste water had transferable resistance to at least one of the drugs ampicillin, chloramphenicol, streptomycin, sulfonamide, or tetracycline as compared to an average of 4% in city sewage. R^+ bacteria in the hospital discharge were also resistant to a broader spectrum of drugs than those in city sewage. In both effluents, the occurrence of fecal *Escherichia coli* among R^+ coliforms was twice as high as among coliforms with nontransferable resistance. Resistance was transferable to *Salmonella typhi*, and such drug-resistant pathogens in the water environment could be of particular concern. The significance of the results with regard to environmental pollution with R^+ bacteria and the dissemination of these organisms is discussed.

Hospital waste water contains relatively low numbers of pathogenic microorganisms (W. O. K. Grabow and E. M. Nupen, Water Res., *in press*). Such effluents have not been investigated for their content of drug-resistant bacteria which cause increasing problems in antimicrobial therapy (1, 6). Selection of resistant organisms can be expected in a hospital environment where drugs are used on a large scale (4, 8, 13, 24).

The known hazard of drug resistance was originally limited. Until recently, only bacteria with nontransferable resistance to one or a few drugs were known (7–9, 27). The currently more alarming type of resistance is mediated by R factors (extrachromosomal genetic elements) which are transferable by conjugation among *Enterobacteriaceae* and other gram-negative organisms such as *Aeromonas* species, *Yersinia pestis*, *Y. pseudotuberculosis*, and *Vibrio cholerae* (16, 25, 27). These factors usually confer resistance to high concentrations of up to eight drugs simultaneously (27). R factors harbored by nonpathogenic intestinal bacteria such as *Escherichia coli* can be transferred to drug-susceptible pathogens such as *Salmonella* or *Shigella* (10, 27). Bacteria which carry R factors (R^+ bacteria) are present in large numbers of patients (1, 4, 6, 14) and healthy individuals (17, 22, 29). R^+ coliform bacteria have been isolated from rivers (19) and coastal bathing waters (20) polluted with sewage plant effluents which contain many of these organisms (19–22). Routine monitoring of R^+ bacteria in sewage may serve to evaluate the incidence of R factors in the general population and to detect changes in the resistance pattern of prevailing R factors (22).

This report compares numbers and some properties of drug-resistant coliforms in the sewage of Pietermaritzburg (population, 113,000) in South Africa and in the sewage of the Edendale General Hospital (1,650 beds) near that city. The hospital waste water is not discharged into the city sewers. The purpose of the study was to evaluate the load of R^+ bacteria in sewage and to determine whether selection for these organisms occurs in the hospital environment. This information is important for consideration of measures to prevent the spread of R^+ bacteria (19–22). Counts of microorganisms in the above effluents have been determined (Grabow and Nupen, *in press*).

MATERIALS AND METHODS

Sewage samples. Hospital sewage and city sewage were sampled simultaneously at about 3-week intervals from January to June 1972 (a total of nine samples from each source) by the methods of Grabow and Nupen (*in press*).

Isolation of resistant bacteria. Saline (0.85%) dilutions of sewage were plated on MacConkey agar (Difco) to obtain the total coliform count and on plates of the same medium contain-

ing one drug (Table 1) to isolate resistant coliforms (19, 22). Unless stated otherwise, incubation was at 37 C. In experiments with sulfonamide, MacConkey agar was replaced by a medium modified to overcome interference from inhibitors (28). This medium consisted of Mueller-Hinton medium (Difco) which contained the selective ingredients of MacConkey agar. Counts were done in duplicate.

Transfer of resistance. Nalidixic acid-resistant mutants of an F⁻ fecal *E. coli* strain, E25, and a *Salmonella typhi* strain, N, were used as recipients. The former was kindly provided by D. R. Woods (29), and the latter was isolated from a patient with typhoid fever. Overnight broth cultures of a drug-resistant coliform (0.1 ml) and a recipient (1 ml) were mixed in 10 ml of Nutrient Broth No. 2 (Oxoid) for conjugation. After overnight incubation, 0.1 ml of each cross was plated on MacConkey agar which contained nalidixic acid (30 μg/ml) and the drug (concentrations in Table 2) to which the potential donor was resistant. Transfer of resistance was confirmed by a second cross in which the recipient with newly acquired resistance was the donor and a mutant of *E. coli* E25 resistant to 250 μg of sodium azide/ml (3) was the recipient (22). Groups of 20 coliforms from every sewage sample were tested for transferable resistance. Each group was resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide, or tetracycline. These drugs were chosen because of their widespread use. The spectrum of resistance transferred was determined (14) by streaking overnight broth cultures of R⁺ recipients on MacConkey agar plates, each supplemented with one drug (concentrations in Table 2).

Fecal *E. coli* strains were identified by growth on MacConkey agar and indole production in tryptophan broth at 44.5 C (Grabow and Nupen, *in press*).

RESULTS

Total coliform counts and numbers of drug-resistant coliforms in hospital and city sewage are listed in Table 1. These results include both transferable and nontransferable resistance because the concentrations of drugs were low (13). Counts of coliforms resistant to Bactrim (25 μg of trimethoprim plus 125 μg of sulfamethoxazole per ml), gentamicin (10 μg/ml), nalidixic acid (25 μg/ml), and nitrofurantoin (200 μg/ml) never exceeded 0.1% of the total coliform count. Table 1 shows that the hospital waste water contained a higher percentage of coliforms resistant to each drug except cephaloridine. Differences between hospital and city sewage were greatest for coliforms resistant to kanamycin, neomycin, or tetracycline. In both hospital and city sewage, resistance was mainly to chloramphenicol, streptomycin, or sulfonamide.

The numbers of coliforms resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide, or tetracycline which transferred resistance to *E. coli* E25 or *S. typhi* N are presented in Table 2. In hospital waste water, 50% of coliform bacteria resistant to at least one of the five drugs transferred their resistance to *E. coli* E25. This figure was only 15% for city sewage. In both effluents, resistance to ampicillin, chloramphenicol, or tetracycline was transferred most frequently. Resistance, especially to chloramphenicol or sulfonamide, was transferred less frequently to *S. typhi* N than to *E. coli* E25. The number of coliforms which infected *S. typhi* N must be regarded as a minimum (22, 27) since the latter organism has not been investigated for

TABLE 1. *Drug-resistant coliforms in hospital and city sewage*

Drug	Concn of drug (μg/ml)	Hospital		City	
		Count[a]	Percent of total	Count[a]	Percent of total
Total coliform count	0	500 (100–1,500)		1,000 (300–1,800)	
Ampicillin	25	179 (110–500)	38 (31–52)	189 (64–200)	20 (10–30)
Cephaloridine	15	70 (13–315)	15 (10–21)	183 (33–350)	19 (10–26)
Chloramphenicol	25	297 (55–825)	62 (55–75)	478 (189–1,000)	45 (30–63)
Kanamycin	25	118 (31–315)	39 (21–52)	20 (11–30)	3 (1–6)
Neomycin	25	151 (40–600)	37 (25–56)	18 (8–52)	2 (1–4)
Oxytetracycline	25	199 (43–870)	48 (27–74)	40 (13–106)	7 (2–15)
Streptomycin	25	348 (73–1,185)	76 (60–93)	216 (80–380)	22 (16–30)
Sulfonamide	25	301 (133–1,245)	73 (60–83)	291 (165–420)	35 (20–55)
Tetracycline	25	137 (31–435)	31 (16–49)	40 (4–87)	4 (1–9)

[a] Count of resistant coliforms in thousands per milliliter. Median count for nine samples followed by the range in parentheses.

recipient ability. Resistance markers were occasionally lost during R factor transfer from coliforms to *S. typhi* N.

Calculations based on results in Tables 1 and 2 show that 26% of all coliform bacteria in the hospital discharge carried R factor resistance to one or more of five drugs (Table 3). This figure was 4% for city sewage. In both effluents, the counts of R^+ coliforms resistant to chloramphenicol were the highest and those resistant to tetracycline were the lowest. The index of R^+/R^- resistant organisms was, however, the same for both drugs. Table 3 also shows that hospital and city sewage did not differ significantly in counts of coliforms with nontransferable resistance to the 5 drugs concerned. The slightly lower number in city sewage is due to its low content of coliforms with nontransferable resistance to streptomycin or tetracycline.

The most frequent patterns of resistance transferred by R^+ organisms to *E. coli* E25 appear in Table 4. The resistance spectrum of coliforms with nontransferable resistance was not investigated. Table 4 shows that in both effluents R factors which confer multiple resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline were encountered most frequently. In hospital sewage, an average 49% of R^+ coliforms transmitted this pattern

TABLE 2. *Number of coliform bacteria with transferable drug resistance in hospital and city sewage*

Drug	Concn of drug (μg/ml)	Hospital[a]				City[a]			
		Resistant coliforms which transferred resistance to				Resistant coliforms which transferred resistance to			
		E. coli E25		*S. typhi* N		*E. coli* E25		*S. typhi* N	
		No.	Percent	No.	Percent	No.	Percent	No.	Percent
Ampicillin	500	108	60	74	41	31	17	27	15
Chloramphenicol	500	97	54	11	6	29	16	1	1
Streptomycin	25	74	41	40	22	20	11	13	7
Sulfonamide	100	56	31	20	11	27	15	7	4
Tetracycline	500	111	62	76	42	32	18	27	15
Average		89	50	44	24	28	15	15	8

[a] The number of resistant coliforms tested for each drug was 180.

TABLE 3. *Total coliform bacteria with transferable and nontransferable drug resistance in hospital and city sewage*[a]

Drug	Coliforms in hospital sewage					Coliforms in city sewage				
	Nontransferable resistance (NR)		Transferable resistance (TR)		Index, TR/NR	Nontransferable resistance (NR)		Transferable resistance (TR)		Index, TR/NR
	Count[b]	Percent of total	Count[b]	Percent of total		Count[b]	Percent of total	Count[b]	Percent of total	
Ampicillin	75	15	115	23	1.5	160	16	40	4	0.3
Chloramphenicol	145	29	165	33	1.1	380	38	70	7	0.2
Streptomycin	225	45	155	31	0.7	190	19	30	3	0.2
Sulfonamide	250	50	115	23	0.5	300	30	50	5	0.2
Tetracycline	60	12	95	19	1.6	30	3	10	1	0.3
Average	151	30	129	26	1.1	212	21	40	4	0.3

[a] Figures calculated from median percentages presented in Tables 1 and 2.
[b] Thousands per milliliter.

TABLE 4. *Patterns of resistance transferred to Escherichia coli E25 by coliforms isolated from hospital and city sewage*

Pattern[a]	Hospital		City	
	No.	Percent	No.	Percent
A, C, S, Su, T	441	49	243	27
C, S, Su, T	117	13	45	5
A, S, Su, T	72	8	153	17
S, Su, T	54	6	117	13
S, Su	36	4	81	9
Other patterns	180	20	261	29

[a] A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfonamide; T, tetracycline.

of resistance compared to an average 27% in city sewage.

The incidence of fecal *E. coli* cells among R^+ coliforms in both hospital and city sewage was twice as high as among coliforms with nontransferable resistance (Table 5). The number of fecal *E. coli* cells in the drug-susceptible coliform population was not investigated.

DISCUSSION

The R^+ incidence of 26% among total coliform bacteria encountered in hospital waste water, as opposed to 4% in city sewage (Table 3), illustrates that selection of R^+ organisms occurred in the hospital. These results represent a quantitative evaluation of R^+ bacteria in the excreta of hospitalized patients and the control urban population. Consequently, they give an indication of the relative number of R^+ bacteria excreted by these populations (21, 22). Datta (4) found no significant increase in the number of patients who carried R^+ bacteria before and during hospitalization. Her results show that there was almost no cross-infection of hospitalized patients with R^+ bacteria. This can be expected because hygienic standards in hospitals are high. Her study confirms that drug treatment does not result in emergence of R^+ bacteria but only in their selection (5, 27).

The above findings illustrate that two factors are involved in the spread of R^+ bacteria. The first is drug therapy which selects for these organisms and the other is their transmission. The latter occurs by environmental pollution with excreta in which selection for R^+ bacteria had taken place. Consequently, the rapid increase in numbers of animals which carry R^+ bacteria among farm stocks exposed to drugs (15, 24) is not due to drug treatment alone but also to unhygienic conditions. Woods et al. (29) similarly observed that the frequency of healthy people carrying R^+ bacteria was higher among populations with low standards of hygiene. Environmental pollution with R^+ bacteria has frequently been noted. The city sewage investigated here contained an average of 40×10^3 R^+ coliforms/ml (Table 3). Many of these organisms survive conventional sewage purification (19, 22), and pollution of various water sources may be expected (20). Resistance was transferable to *S. typhi*, and such drug-resistant pathogens in the water environment could be of particular concern in bathing waters. R^+ bacteria have also been isolated from milk, meat (24, 26), and sausages (18). Steps to prevent environmental pollution with R^+ bacteria are therefore as important for R factor control as better drug utilization (2) or the use of new

TABLE 5. *Incidence of fecal Escherichia coli among drug-resistant coliform bacteria isolated from hospital and city sewage*

Drug	Hospital				City			
	Nontransferable resistance		Transferable resistance		Nontransferable resistance		Transferable resistance	
	No. tested	No. of fecal *E. coli*	No. tested	No. of fecal *E. coli*	No. tested	No. of fecal *E. coli*	No. tested	No. of fecal *E. coli*
Ampicillin	50	8 (16%)	56	24 (43%)	86	4 (5%)	16	9 (56%)
Chloramphenicol	43	11 (26%)	53	17 (32%)	53	20 (38%)	27	20 (74%)
Streptomycin	73	13 (18%)	26	10 (38%)	96	9 (9%)	9	1 (11%)
Sulfonamide	80	10 (13%)	29	9 (31%)	95	9 (9%)	14	0 (0%)
Tetracycline	35	5 (14%)	63	17 (27%)	71	24 (34%)	39	20 (51%)
Average	56	9 (17%)	45	15 (34%)	80	13 (19%)	21	10 (38%)

drugs (7). Since polluted water plays a major role in the transmission of microorganisms (20, 23), improved purification of sewage should receive special attention.

In contrast to the notable difference in numbers of R^+ organisms, the counts of coliforms with nontransferable resistance did not differ significantly in hospital and city sewage (Table 3). This may explain the exceptionally low counts of cephaloridine-resistant coliforms in the hospital discharge, because it is the only drug among those in Table 1 for which transferable resistance has not been detected (5). The inability of some coliforms to transfer resistance to *S. typhi* and segregation of determinants during transfer (Table 2; 19, 20, 27) may explain the low incidence of R^+ *S. typhi* cells resistant to chloramphenicol (11, 12). R^+ bacteria in the hospital discharge were resistant to a broader spectrum of drugs than those in city sewage (Table 4). This shows that exposure to drugs not only selects for transferable resistance but also for R factors with more resistance determinants. The high incidence of streptomycin, sulfonamide, and tetracycline markers carried by R^+ bacteria in both hospital and city effluents (Table 4) confirms that resistance to these drugs is common (4, 19, 22).

Our counts of R^+ coliforms in city sewage were higher than those recorded recently in Britain (19) and the United States (21). This indicates that the occurrence of R^+ organisms may be increasing. The incidence of fecal *E. coli* cells in both hospital and city sewage was twice as high as among coliforms with nontransferable resistance (Table 5). Consequently, R^+ *E. coli* may serve as a marker for the identification of sources of pollution in contaminated waters (21).

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