

Drug-Resistant Bacteria in Continental Shelf Sediments

SAGAR M. GOYAL^{1*} AND WILLARD N. ADAMS²

Department of Veterinary Diagnostic Investigation, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55108,¹ and Northeast Technical Services Unit, Food and Drug Administration, Davisville, Rhode Island 02854²

Received 16 January 1984/Accepted 19 July 1984

Fecal indicator bacteria were isolated from water and sediment samples obtained from a sewage sludge disposal site in the Middle Atlantic. Some were found to be resistant to several antimicrobial agents. Isolation of these microbes 30 months after cessation of sludge dumping indicates their survivability in the environment.

Pollution of coastal and oceanic environments is not only esthetically displeasing but also potentially hazardous to human health. The problem seems to be more acute for the densely populated northeast United States. In 1973, the U.S. Environmental Protection Agency designated a sewage sludge dumpsite 40 nautical miles (74 km) off the Delaware-Maryland coast in the Middle Atlantic Bight (Fig. 1). By 1979, this dumpsite had received 305×10^6 kg of sewage sludge from the cities of Philadelphia, Pa., and Camden, N.J. The dumpsite occupies an area of 50 km² and is located in water 45 to 60 m deep. Dumping of sludge at the Philadelphia dumpsite ceased on 25 November 1980. More than 20 oceanographic cruises were made to the site from 1973 to 1983 to assess the environmental effects of dumping (7). The present report concerns the isolation of drug-resistant coliform bacteria from water and sediments 30 months after sludge dumping ceased at this site.

During a cruise to the Philadelphia dumpsite in June 1983, samples of water and bottom sediments were collected and examined for the presence of indicator bacteria. Sediments were collected with a Smith-McIntyre bottom sampler. The top 5-mm layer of sediment was removed, and most-probable numbers of total and fecal coliforms were determined according to *Standard Methods* (1) as described previously (3, 8). The membrane filter technique was used to enumerate fecal indicator bacteria from 100-ml samples of bottom-hinge water samples. Bacteria isolated on-board were later identified with the API 20E system (Analytab Products, Plainview, N.Y.). Resistance of these bacteria to 13 different antibiotics was determined as described previously (4, 5).

Samples were collected from several stations located at the Philadelphia dumpsite. Four of 24 water samples and 6 of 63 sediment samples yielded total or fecal coliform bacteria or both. Species identification of bacterial isolates and resistance determinants carried by them are shown in Table 1. Of 10 isolates, only 2 were completely sensitive to all antimicrobial agents tested. The remaining eight isolates carried between one and six resistance determinants. At least three isolates were resistant to three or more antibiotics. These multiple resistance strains are the most likely to carry R-plasmids which are transferable to other bacteria.

Isolation of drug-resistant coliforms from the Philadelphia dumpsite 30 months after the cessation of sludge dumping indicates that these bacteria are capable of extended survival in the marine environment. After the disposal of sludge in

water, the bacteria are greatly reduced in numbers as a result of dilution and die-off. However, bacteria contained in sludge particulate matrix reach the ocean bottom and become a part of bottom sediments which are maintained at a constant temperature of ca. 7°C. We believe that the protective sludge matrix and low temperature are contributing environmental conditions that apparently sustain the viability of these bacteria. It has previously been shown that bacteria survive longer in the presence of sediments than in seawater alone (2).

Drug-resistant bacteria have been isolated from sediments of another sewage sludge disposal site located in the New York Bight apex (6, 9, 10). Those studies indicated that bottom sediments may act as long-term reservoirs of drug-resistant bacteria which may be reintroduced into the environment by storms, dredging, boating, and other activities.

Resistant bacteria may be concentrated by filter-feeding shellfish which inhabit waters overlying the dumpsites. Consumption of such shellfish by humans may result in colonization of the human gut by these bacteria. Although not pathogenic themselves, these bacteria may transfer their

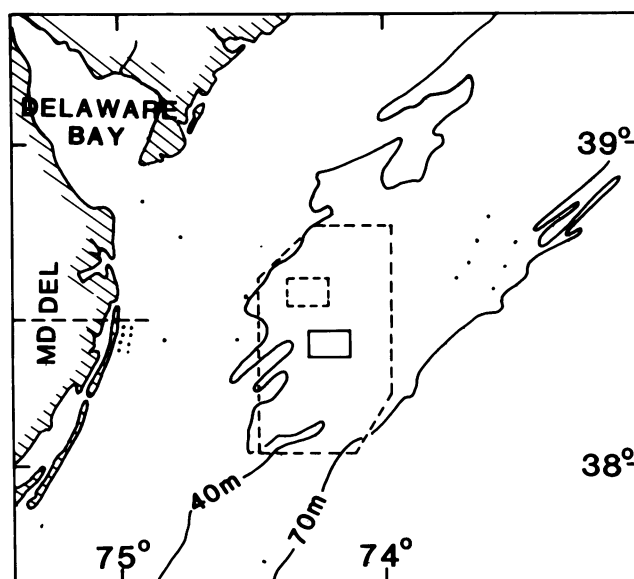


FIG. 1. Philadelphia sewage sludge dumpsite shown as a solid rectangle between 40- and 70-m isobaths. Rectangle with broken lines is an acid-waste site which is no longer in use.

* Corresponding author.

TABLE 1. Drug resistance pattern of bacteria isolated from sewage sludge disposal site

Station no.	Sample type ^a	Bacteria	Antibiotic resistance ^b												
			AM	C	FR	CF	GM	N	VT	Tri	Px	SP	Te	TS	KN
11	Sed	<i>Enterobacter cloacae</i> ^c	R	S	S	R	S	S	S	S	S	S	S	S	S
20	Sed	<i>Klebsiella pneumoniae</i>	R	S	S	S	S	S	S	S	S	S	S	S	S
307	Sed	<i>Citrobacter freundii</i>	R	S	S	R	S	S	S	S	S	S	S	S	S
439	W	<i>Proteus vulgaris</i>	R	R	R	R	S	S	S	S	R	S	R	S	S
G19	W	<i>Aeromonas hydrophila</i>	R	S	S	S	S	S	S	S	S	S	S	S	S
T15	Sed	<i>Klebsiella oxytoca</i> ^c	R	S	S	R	S	S	S	S	S	S	S	R	S
T18	Sed	<i>Klebsiella pneumoniae</i> ^c	S	S	S	S	S	S	S	S	S	S	S	S	S
OC-2	W	Centers for Disease Control group 5 E-1	R	S	R	R	S	S	S	S	S	R	R	S	S
OC-7	Sed	<i>Escherichia coli</i> ^c	S	S	S	S	S	S	S	S	S	S	S	S	S
OC-10	W	<i>Enterobacter agglomerans</i>	S	S	S	S	S	S	S	S	S	R	S	R	S

^a W, Water sample; Sed, sediment sample.

^b S, Sensitive; R, resistant; AM, ampicillin; C, chloramphenicol; FR, furadantin; CF, cephalothin; GM, gentamicin; N, neomycin; VT, vetasulid; Tri, trimethoprim; Px, polymyxin; SP, spectinomycin; Te, tetracycline; TS, triple sulfa; and KN, kanamycin.

^c Fecal coliforms.

resistance to already colonized bacteria or other human pathogens. Further studies are necessary to determine the occurrence of transferable drug-resistant bacteria in fish and shellfish in and around the dumpsite. The dumpsite should also be monitored regularly to determine how long these bacteria can survive under existing field conditions.

This study was supported in part by a grant from the National Oceanic and Atmospheric Administration (NA83AA-D00050).

We thank Don Lear, Marria O'Malley, Patricia Charnis, Mildred Hakomaki, and the crew of *Antelope* for help in sample collection and analysis.

LITERATURE CITED

1. American Public Health Association. 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.
2. Gerba, C. P., and J. S. McLeod. 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. Appl. Environ. Microbiol. 32:114-120.
3. Goyal, S. M., C. P. Gerba, and J. L. Melnick. 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. Appl. Environ. Microbiol. 34:139-149.
4. 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.
5. Goyal, S. M., and A. W. Hodley. 1979. Salmonellae and their associated R-plasmids in poultry processing wastes. Rev. Microbiol. 10:50-58.
6. Koditschek, L. K., and P. Guyre. 1974. Antimicrobial-resistant coliforms in New York bight. Mar. Pollut. Bull. 5:71-74.
7. National Oceanic and Atmospheric Administration. 1983. Report to the Congress on ocean pollution, monitoring and research, October 1981 through September 1982. Department of Commerce, Washington, D.C.
8. O'Malley, M. L., D. W. Lear, W. N. Adams, J. Gaines, T. K. Sawyer, and E. J. Lewis. 1982. Microbial contamination of continental shelf sediments by wastewater. J. Water Pollut. Control Fed. 54:1311-1317.
9. Stewart, K. R., and L. K. Koditschek. 1980. Drug resistance transfer in *Escherichia coli* in New York Bight sediment. Mar. Pollut. Bull. 11:130-133.
10. Timoney, J. F., J. Port, J. Giles, and J. Spanier. 1978. Heavy-metal and antibiotic resistance in the bacterial flora of sediments of New York Bight. Appl. Environ. Microbiol. 36:465-472.