ANTIBIOTIC RESISTANT BACTERIA

FROM

NEW YORK HARBOR

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ABSTRACT

Water samples were collected from thirteen separate sites in New York Harbor. Samples were plated on eosin methylene blue agar and forty-two separate isolates were developed by standard microbial isolation techniques. The isolates were subjected to macromolecular testing and the API 20E identification system for Enterobacteriaceae. Six separate gram negative bacilli were identified. The presences of Escherichia, Enterobacter, Serratia and Salmonella were confirmed by the API identification procedures and Klebsiella and Proteus were tentatively identified. All six isolated organisms were tested against a spectrum of forty-seven antimicrobial agents used in therapeutic dosages. The isolated organisms showed marked resistance to all the agents tested except sulfathiazole, sufisoxazole, triple sulfa drugs and sulfamethoxazole with trimethoprim.

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INTRODUCTION

In recent years there has been a steady increase in the number of infectious diseases caused by antibiotic resistant bacteria. Many of these infections have spread through the human population, with almost epidemic speed, especially in urban areas. The development of these new "superbugs" has been attributed to society's abuse of antibiotics, poor sewage treatment and the microbes' incredible ability to mutate in relatively short periods of time.

Since the introduction of antibiotic drugs in the 1940s, the use of these antimicrobial agents has become commonplace in medical and industrial areas. It is this overuse, a result of the popular myth that antibiotics are "cure alls", that has led into investigations of the mechanisms by which harmless bacterial strains have developed the means of resisting the effects of these drugs. It has been seen that as more bacteria are exposed to and destroyed by antibiotics, there are still a certain number that will survive this attack (Cohen, 1992; Dixon, 1986). These resistant cells can then flourish and multiply in their environment.

Antibiotic resistant bacteria owe their ability to counteract antimicrobial drugs to rapid evolution through natural selection. Once susceptible cells are destroyed by an antibiotic, resistant cells, no matter their numbers, can begin to reproduce. These microbes replicate frequently

over a short period of time. During these many replications, the genetic blueprint of the cell's DNA may mutate, contributing further to drug resistance. This trait is then passed down to other generations of cells. If a new drug is introduced, the cycle of natural selection and evolution begins again, unless all previously resistant cells are successfully destroyed (Neu, 1992; Richmond, 1983).

Antibiotic resistant can also be transferred between two different strains of bacteria, that may cohabitate, through the process of conjugation. In conjugation, the genetic components of two bacterial forms are exchanged. It is during conjugation that extrachromosomal fragments of DNA, called plasmids, can be exchanged as well. The plasmids responsible for antibiotic resistance are called R-factors and are often exchanged between resistant and non-resistant bacterial strains (Godwin and Slater 1979). This action dramatically expands the distribution of the original resistant factors and also increases the variability of those resistant factors.

The Enterobacteriaceae family of bacteria is composed of gram negative and oxidase negative bacilli which can exhibit aerobic or facultative anaerobic respiration. These microbes also produce acid fermentatively from glucose and reduce nitrates to nitrites. The subgroup of this family, the coliforms, normally inhabit soil, water, decaying matter

and fecal matter, and the large intestine of both humans and animals (Holt, 1984). The presence of coliforms in waterways, due to hospital, commercial and residential dumping, often indicates the degree of water pollution (Farmer et al., 1985). Coliform bacteria are classified as opportunistic organisms rather that intestinal pathogens; they can infect any body tissue, including that of the intestinal tract, when the host organism is in a weakened state. The coliforms are also known to cause a majority of nosocomial infections present today.

It was once believed that the plasmid transfer of the coliform bacteria only occurs in the alimentary tract of those mammals that ingest R-factor organisms in food or antibiotics that selected the resistant bacteria. Evidence has arisen to support the concept of plasmid transfer occurring outside these physiological conditions (Davies and Round 1972). The largest concentrations of coliforms can usually be found near the waters surrounding sewage treatment plants (Kelch and Lee, 1978; Murray et al., 1984). These local waterways provide the perfect conditions for fecal coliforms with R-factors to multiply and then transfer their plasmids to other strains. Therefore, a mixed coliform population sharing a common environment can exhibit similar degrees of antibiotic resistance and other changes in microbial physiology.

Antimicrobial agents have been a remarkable adjunct to

medical science for almost sixty years. Antibiotics have been produced since the mid 1940s by pharmaceutical companies as part of the constant battle against microbial forms of disease. Antibiotics represent a large spectrum of the chemotherapeutic agents used for human and veterinary purposes (Garrod and O'Grady, 1973). Pharmaceutical companies in the past four decades have developed increasingly stronger antimicrobial compounds in order to keep abreast of the development of resistant strains of microorganisms (Krause, 1992; Kuntz, 1992). Their strategies work for a finite period of time and then the microbes overrun the effectiveness of the new agents. Antibiotic resistance requires constant surveillance for troublesome microbes and has resulted in the development of laboratory approved testing procedures (Bauer et al., 1966).

The Enterobacteriaceae represent a group of bacteria which inhabit various ecological niches, including the colon region of humans and animals. They were once considered harmless microbes or saprophytes. They are a widely diverse group and their diversity is rapidly expanding due to plasmid transfer of genetic information (Ewing, 1986). Antibiotic resistant Enterobacteriaceae are often found in several areas, such as, hospitals - a source of nosocomial infections, the human intestinal tract and areas associated with sewage disposal. The continued development of these resistant microbial forms represents a serious medical

problem for the ever increasing numbers of immunodeficient individuals in our populations.

The goal of this project was to determine the presence and the horizontal range of antibiotic resistant bacteria, especially members of the family Enterobacteriaceae. The specimens used in this investigation were coliforms isolated from the local waters of New York Harbor. Many of the selected sites were listed in an article in The New York Daily News, issued July 7, 1986, as being the most heavily polluted waterway areas due to the direct discharge of sewage. This study set out to confirm the hypothesis that the overuse of antibiotics has caused bacteria present in New York City waters to become antibiotic resistant.

METHODS

Collection Sites

Thirteen separate sites were selected for sample selection in New York Harbor. Sites selection was based on previous ministudies conducted in advanced biology courses at St. Francis College, which involved analyzing the degree of coliform pollution present in local waters.

Samples were collected with standard field equipment in the last week of May and the first week of June during conditions of moderate rainfall. The meteorological conditions provided a near optimum state for sample collection. Effluent from the combined storm drain sewer system and the sewage treatment plants produced elevated levels of coliform organisms in the waters of the harbor.

At each site, water samples were collected with either a Van Dorn water sample or a sterile two-liter plastic bag depending on the accessibility of the site. Temperature, oxygen and pH values were measured at each site with portable field equipment. Samples were transported back to the laboratory and kept under refrigeration prior to initial platings on media. Preliminary sample platings were carried out 12 to 24 hours after sample collection. The collection sites and related field data are listed in Table 1.

Isolation Procedure

Initial platings were conducted under sterile conditions with eosin methylene blue agar (EMB). Bacterial colonies generally developed after incubation at 35° C, within 18 to 24 hours. Isolated colonies were aseptically transferred to trypticase soy broth (TSB) and inoculated at 35° C for 24 hours to begin initial isolates. Seventeen separate isolates were developed by this procedure. With plating on various differential, selective media, 42 separate isolates were obtained from the raw environmental samples. The forty-two isolates were tested against a comprehensive series of macromolecular tests for biochemical characterization.

Gram Stain Procedure

Samples of each isolate were smeared on glass slides and allowed to air dry. After 30 minutes the slides were flame fixed and prepared for staining. A standard gram stain procedure was conducted on each sample to separate gram negative bacilli from other samples. Gram stain analysis was conducted periodically to insure that all test samples were gram negative bacilli.

Standard Cultures

Standard cultures used for control/comparison testing were bacterial forms from the American Type Culture Collection (ATCC). The organisms used were: *Escherichia coli*, *Enterobacter aerogenes*, *Serratia marcescens* and

Table 1 Collection Sites

Site/Location	Code	Temp. (°C)	O_2 Level (ppm.)	pH
1. Williamsburg Bridge/East River	ER/WB	17.0	5.9	7.73
2. Flushing Bay	FB	18.0	5.4	7.78
3. Newton Creek	NC	17.0	6.2	8.06
4. Hudson River/ World Trade Center	HR/WTC	18.0	5.9	8.36
5. Breezy Point	BP	18.0	9.4	7.76
6. Hudson River/ 79th Street	HR/79	18.0	6.6	7.70
7. Atlantic Avenue	AA	18.5	5.8	8.00
8. Plumb Beach	PB	18.0	9.0	7.68
9. 69th Street Pier/ Bay Ridge	69/BR	15.0	6.9	8.30
10. Broad Channel/ Queens	BC/Q	20.0	10.2	7.97
11. Fort Hamilton/ Verrazano-Narrows Bridge	VB/FH	16.0	7.2	7.75
12. Gowanus Canal	GC	18.0	6.2	9.33
13. Great Kills/ Staten Island	GK/SI	20.5	7.8	7.81

Salmonella typhimurium.

Glassware

All glassware was washed Alconox, brush scrubbed and rinsed with tap water. All cleaned glassware was baked at 400 C for 24 hours in a hot air oven. Screw-cap borosilicates test tubes with caps were used for all experiments. All agar experiments were conducted in standard sterile Petri dishes, except the antibiotic assays, which were conducted in 20 x 150 mm plastic Petri dishes.

Experimental Growth Conditions

All experiments were conducted in a Napco or a Lab-Line Ambi Hi non-carbon dioxide incubator set at 35° C ± 1° C for 18 to 24 hours, except in experimental procedures that required longer incubation times.

Miscellaneous Equipment

All prepared media were sterilized in a American Sterilizer autoclave. Heat sensitive sterile indicator tape was used to insure the integrity of each sterilization procedure in both media preparation and destruction of completed experiments. All media were prepared with Corning pH meter and deionized water. All Gram stain slides were read under oil immersion with a Bausch and Lomb Balplan microscope.

Media, Reagents and Tests

All artificial media and reagents for experiments were prepared with J.T. Baker reagent grade chemicals.

Dehydrated microbial media, produced by Difco or BBL, were used for experimental procedures. Carbohydrates and other bio-organic substances were supplied by ICN (Nutritional Biochemical Corporation) chemicals. All media and reagents were prepared with fresh deionized water, autoclaved when appropriate, and stored under refrigeration. Several selected media (Hektoen Enteric Agar (HEA), Salmonella-Shigella Agar (SS), Bismuth Sulfite Agar (BSA) and Xylose-Lysine-Deoxycholate Agar (XLD) were freshly prepared without autoclaving and refrigerated briefly prior to inoculation and incubation. These media would have been destroyed or reduced in growth effectiveness if they had been autoclaved. Microbial media were prepared in accordance with directions listed in the Difco Manual (Difco, 1984). All media used in the experimental procedure is listed in Table 2.

Enterotube II Procedure was used to identify the various isolated colonies from the EMB agar plates. This system is a standardized microcompartment version of the conventional procedures used for microbial identification. An isolated colony is used to inoculate the entire tube. The tube is incubated at 35• C to 37• C for 18 to 24 hours. The Enterotube II system contains microamounts of prepared media, which when inoculated will give results similar to macromolecular testing. The biochemical tests carried out by this system are as follows: (1) glucose, (2) lysine decarboxylase, (3) ornithine, (4) hydrogen sulfide/indole

Table 2 Experimental Media

Media/Code	Function
Bismuth Sulfite Agar (BSA)	isolation,differentiation
Brilliant Green Agar (BGA)	isolation,differentiation
E C Medium (EC)	differentiation
Eosin Methylene Blue Agar (EMB)	differentiation
Hektown Enteric Agar (HEA)	isolation,differentiation
Kligler Iron Agar (KIA)	identification
Methyl Red - Voges-Proskauer (MR - VP)	identification, differentiation
MacConkey Medium (Mac)	differentiation
Phenol Red Carbohydrate characterization (PRCM)	biochemical medium
SIM Medium (SIM)	biochemical characterization
Salmonella-Shigella Agar (SS)	selective isolation
Simmon's Citrate Agar (Cit)	identification, differentiation
Tetrathionate Broth (TTB)	isolation, enrichment
Triple Sugar Iron Agar (TSIA)	biochemical characterization
Trypticase Soy Broth (TSB)	general growth
Xylose-Lysine Deoxycholate Agar (XLD)	differentiation
Urea Broth (UB)	biochemical characterization
Oxidase Test	biochemical characterization

Table 2 Experiemental Media (continued)

Media/Code	Function
Catalase Test	biochemical characterization
Mueller Hinton Medium (MH)	antibiotic testing
Enterotube II System	biochemical characterization
A.P.I. 20E System	biochemical characterization

formation, (5) adonitol, (6) sorbitol, (7) arabinose, (8) Voges-Proskauer, (9) dulcitol/phenylalanine deaminase, (10) urea and (11) citrate.

The A.P.I. 20E system identified organisms obtained from the isolated colonies on the EMB agar plates. This system is a standardized microtube version of the conventional procedures used for the identification of Enterobacteriaceae and other gram-negative bacteria. An isolated colony is suspended in sterile saline and the resulting bacterial suspension is inoculated into the microcupule in the test strip. The test strip is incubated at 35° C in a non-carbon dioxide incubator for 18 to 24 hours. The A.P.I. 20E system contains microamounts of prepared media, which when inoculated will give results similar to the macromolecular testing. The biochemical tests carried out by this system are as follows:

(1) orthonitrophenolgalactosidase, (2) arginine dehydrolase, (3) lysine decarboxylase, (4) ornithine decarboxylase, (5) citrate, (6) hydrogen sulfide, (7) urease, (8) tryptophan deaminase, (9) indole, (10) Voges-Proskauer, (11) gelatin, (12) glucose, (13) mannitol, (14) inositol, (15) sorbitol, (16) rhamnose, (17) saccharose/sucrose, (18) melibiose, (19) amygdaline and (20) arabinose.

Antibiotic Sensitivity Testing

Sensitivity/resistance to a total of 47 antimicrobial agents, which include a wide spectrum of antibiotics and

sulfur drugs were determined with BBL sensi-disc test discs by the Kirby-Bauer method. The antimicrobial agents were added under aseptic conditions to Petri dishes containing Mueller Hinton agar, which had been streaked with a diluted broth cultures of each isolate. The plates were incubated for 18 to 24 hours at 35° C in a non-carbon dioxide incubator. The plates were read after the proper incubation period. The diameter of the zone of growth inhibition in millimeters was measured with a ruler. The diameter of each of the zones of inhibition of the water sample isolates was compared with the BBL Microbiology Systems Susceptibility Chart for gram-negative organisms in order to determine resistance or susceptibility to a particular antibiotic. An interpretation of susceptibility or resistance is dependent on the size of the zone of inhibition. Table 3 lists the antimicrobial agents that were tested against the isolated bacteria.

Table 3 Antimicrobial Agents

Antimicrobial Agent	Code	Concentration
Amdinocillin	AMD-10	10 mcg.
Amikacin	AN-30	30 mcg.
Ampicillin	AM-10	10 mcg.
Amoxicillin	AMC-30	20/10 mcg.
Bactracin	B-10	10 units
Carbenicillin	CB-100	100 mcg.
Cefaclor	CEC-30	30 mcg.
Cefotetan	CTT-30	30 mcg.
Cefoxitin	OX-30	30 mcg.
Ceftriaxone	CRO-30	30 mcg.
Cephaloridine	CD-30	30 mcg.
Cephalothin	CF-30	30 mcg.
Choramphenicol	C-30	30 mcg.
Chlortetracycline	A-30	30 mcg.
Clindamycin	CC-2	2 mcg.
Cloxacillin	CX-1	1 mcg.
Colistin	CL-10	10 mcg.
Doxycycline	D-30	30 mcg.
Erythromycin	E-15	15 mcg.
Furazolidone	FX-100	100 mcg.
Gentamicin	GM-10	10 mcg.
Imipenem	IPM-10	10 mcg.

Table 3 Antimicrobial Agents (continued)

Antimicrobial Agent	Code	Concentration
Kanamycin	K-30	30 mcg.
Methicillin	DP-5	5mcg.
Mexlocillin	MZ-75	75 mcg.
Nafcillin	NF-1	1 mcg.
Nalidixic Acid	NA-30	30 mcg.
Neomycin	N-30	30 mcg.
Nitrofurantoin	F/M-300	300 mcg.
Nitrodurazone	Fc-100	100 mcg.
Novobicin	NB-30	30 mcg.
Oxacillin	OX-1	1 mcg.
Oxolinic Acid	OA-2	2 mcg.
Oxytetracycline	T-30	30 mcg.
Penicillin	P-10	10 units
Polymyxin B	B-300	300 units
Rifampin	RA-5	5 mcg.
Streptomycin	S-10	10 mcg.
Sulfamethizole	THE-.25	0.25 mg.
Sulfamethoxazole with Trimethoprim	SXT	1.25 mcg. 23.75 mcg.
Sulfathiazole	ST-.25	0.25 mcg.
Sulfisoxazole	G-0.25	0.25 mg.
Tetracycline	Te-30	30 mcg.
Tobramycin	NN-10	10 mcg.

Table 3 Antimicrobial Agents (continued)

Antimicrobial Agent	Code	Concentration
Trimethoprim	TMP-5	5 mcg.
Triple Sulfa	SSS- 1.0	1.0 mg.
Vancomycin	Va-30	30 mcg.

RESULTS

A single set of water samples was collected from each of the thirteen sampling sites until all sampling had been completed. The meteorological conditions of moderate rainfall during the collection period provided a near optimum state for sample collection. The samples were stored under refrigeration until preliminary plating was conducted.

Thirteen separate initial isolates were developed by the preliminary plating procedure. Serial replication of this procedure using differential agar sources eventually produced 42 separate isolates from the 13 different sample sites. The 42 isolates were tested with the gram stain procedure and a comprehensive series of macromolecular test (SIM, Cit, TSIA, KIA, MR-VP, PRCM and UB) to remove bacteria that were not gram negative Enterobacteriaceae. The EC medium gave positive evidence of coliform bacteria in all isolates. The phenol red carbohydrate medium, which was used to conduct fermentation tests associated with lactose, glucose and sucrose as substrates gave positive production of acid and gas for glucose and sucrose and only acid production for lactose. The urea broth gave positive reaction for a number of isolates. These isolates were separated for presumptive identification as members of the genera Proteus and Klebsiella.

All isolates and controls gave a positive catalase test. Fifteen percent of the isolates gave a negative oxidase reaction. These isolates were screened through a gram stain reaction and non-Enterobacteriaceae isolates were removed from the sample. Fifty percent of all isolates gave a positive hydrogen sulfide reaction to the SIM medium. S. typhimurium was the only control organism that gave a positive hydrogen sulfide reaction.

The standard IMViC procedure was conducted to achieve proper identification of the isolates. The results of the IMViC tests are shown in Table 4. This testing procedure, which is used for differentiation of Enterobacteriaceae provided six separate tentative groupings from the original 42 isolates. These six separate groups formed the basis for all future testing modes.

The Kligler Iron Agar (KIA) and the Triple Sugar Iron Agar (TSIA) were used as differential media that would provide further presumptive identification of the isolates. The six separate groups of isolates gave characteristic reactions in the Kligler Iron Agar (KIA) and Triple Sugar Iron Agar (TSIA) for coliform bacteria. The butt or base of the media and the slant of the media showed characteristic red color (alkaline) or yellow color (acid) reactions. Open spaces in the butt of the agar indicated gas production and black coloration in the medium indicated the presence of hydrogen sulfide. The Kligler Iron Agar (KIA) studies are

Table 4 IMVIC Studies

Presumed Isolate Group	Idole Test	Methyl Red Test	Voges-Proskauer Test	Citrate Test
Escherichia Group	+	+	-	-
Enterobacter Group	-	-	+	+
Serratia Group	-	-	+	+
Salmonella Group	-	+	-	+
Klebsiella Group	+	+	+	+
Proteus Group	–	+	-	+

+ = positive reaction from isolates

- = negative reaction form isolates

+ = variable reaction form isolates

shown in Table 5 and the Triple Sugar Iron Agar (TSIA) studies are shown in Table 6.

The six groups of isolates were inoculated into Tetrathionate Broth (TTB), which was used for the enrichment of Salmonella organisms. Salmonella was present originally in all six isolate groups; but was successfully removed by the use of seven separate media, which were used for differential analysis and isolation of each isolate organism. Escherichia, Enterobacter, Serratia, Salmonella, Klebsiella and Proteus were identified through colony characteristics on each of the differential media. Control organisms demonstrated similar patterns to the isolate groups. Table 7 and 8 shows the reaction of the isolates to the various differential media.

An Enterotube II analysis was conducted on the isolates to provide confirmed identification of the organisms. The results of this procedure were inconclusive. They did not provide a complete identification of any of the isolates. The A.P.I. 20E system, which is an analytical profile procedure for Enterobacteriaceae, provided confirmed identification of Escherichia, Enterobacter, Serratia, Salmonella and strong tentative identification of Klebsiella and Proteus. This identification procedure with its computer data base is far superior to other similar testing modes. There was very strong correlation in the biochemical testing procedures of this test with the control organisms.

Table 5 Kligler Iron Agar Studies

Presumed Isolate Group	Butt	Slant	Gas	H_2S
Escherichia Group	acid	acid	+	-
Enterobacter Group	acid	acid	+	-
Serratia Group	alkaline	acid	+	-
Salmonella Group	alkaline	acid	+	-
Klebsiella Group	acid	acid	+	+
Poteus Group	alkaline	acid	-	+

+ = positive reaction from isolates

- = negative reaction from isolates

Table 6 Triple Sugar Iron Agar Studies

Presumed Isolate Group	Butt	Slant	Gas	H_2S
Escherichia Group	acid	acid	+	-
Enterobacter Group	acid	acid	+	-
Serratia Group	acid	alkaline	-	-
Salmonella Group	alkaline	acid	+	+
Klebsiella Group	acid	acid	+	-
Proteus Group	acid	acid	+	+

+ = positive reaction from isolates

- = negative reaction from isolates

Table 7 Differential Agar Analysis

Presumed Isolate Group	BGA	BSA	EMB
Escherichia Group	yellow green	brownish green	purple, greenish metallic sheen
Enterobacter Group	yellow green	brownish green	pink colonies
Serratia Group	yellow green	brownish green	greenish metallic sheen
Salmonella Group	pink-white colonies	black metallic sheen	colorless
Klebsiella Group	yellow green	brownish green	green metallic sheen
Proteus Group	yellow green	brownish green	green metallic sheen

BGA = Brilliant Green Agar

BSA = Bismuth Sulfide Agar

EMB = Eosin Methylene Blue Agar

Table 8 Differential Agar Analysis

Presumed Isolate Groups	H.E.A.	Mac	SS	XLD
Escherichia Group	orange/	colorless bile ppt.	colorless red center	yellow
Enterobacter Group ppt.	salmon-orange	colorless red center	cream-pink	yellow/ black
Serratia Group	greenish	pink-red	colorless	yellow
Salmonella Group	greenish blue	colorless	colorless	red/black center
Klebsiella Group	greenish blue	colorless	coloreless	yellow
Proteus Group	greenish/ black center	colorless	colorless black center	yellow/ black center

HA = Hektoen Agar

Mac = MacConkey Agar

SS = Shigella Salmonella Agar

XLD = Xylose-Lysine Deoxycholate Agar

Table 9 shows the results of the A.P.I. 20E analysis of Escherichia and Enterobacter. Table 10 shows the results of the A.P.I. 20E analysis of Serratia and Salmonella. Table 11 shows the results of the A.P.I. 20E analysis of Klebsiella and Proteus.

Antimicrobial tests with a total of 47 separate compounds were conducted according to the standard Kirby-Bauer method. The list of discs, their codes and respect agent concentrations are given in Table 3. All isolates showed resistance to the test antibiotics and some degree of intermediate or minimal susceptibility to several sulfur drugs. Sulfathiazole, sulfisoxazole, triple sulfa drug and sulfamethexazole with trimethoprim were the only antimicrobial substances that demonstrated any action against the isolated organisms. Table 12 shows the spectrum of action of antimicrobial agents against Escherichia and Enterobacter. Table 13 shows the spectrum of action of antimicrobial agents against Serratia and Salmonella. Table 14 shows the spectrum of action of antimicrobial agents against Klebsiella and Proteus.

Table 9 API 20E Analysis

Test Code	Escherichia isolate	Escherichia control	Enterobacter isolate	Enterobacter control
OPNG	+	+	+	+
ADH	-	-	-	-
LDH	+	+	+	+
ODC	+	+	+	+
CIT	-	-	+	+
H_2S	-	-	-	-
URE	-	-	+	-
TDA	-	-	+	+
IND	+	+	-	-
VP	-	-	+	+
GEL	-	-	+	-
GLU	+	+	+	+
MAN	+	+	+	+
INO	-	+	+	+
SOR	+	+	+	+
RHA	+	+	+	+
SAC	-	+	+	+
MEL	+	+	+	+
AMY	-	-	+	-
ARA	+	+	+	+
OXI	-	-	-	-

Table 10 API 20E Analysis

Test Code	Serratia isolate	Serratia control	Salmonella isolate	Salmonella control
OPNG	+	+	-	-
ADH	-	+	-	-
LDH	+	+	+	+
ODC	+	+	+	+
CIT	+	+	+	+
H_2S	-	-	+	+
URE	+	-	-	-
TDA	-	-	+	+
IND	-	-	-	-
VP	+	+	-	-
GEL	+	-	-	-
GLU	+	+	+	+
MAN	+	+	+	+
INO	+	+	+	-
SOR	+	+	+	+
RHA	-	+	+	+
SAC	+	+	+	-
MEL	-	-	+	+
AMY	+	+	+	-
ARA	-	-	+	+
OXI	-	-	-	-

Table 11 API 20E Analysis

Test Code	Klebsiella	Proteus
OPNG	+	-
ADH	-	-
LDH	+	-
ODC	-	+
CIT	+	+
H_2S	-	+
URE	+	+
TDA	+	+
IND	+	-
VP	+	-
GEL	-	+
GLU	+	+
MAN	+	-
INO	+	-
SOR	+	-
RHA	+	-
SAC	+	-
MEL	+	-
AMY	+	-
ARA	+	-
OXI	-	-

Table 12 Antimicrobial Susceptibility Patterns

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Escherichia	Enterobacter
Amdinocillin	>16	R	R
Amikacin	>17	R	R
Ampicillin	>17	R	R
Amoxicillin	>18	R	R
Bactracin	>13	R	R
Carbenicillin	>23	R	R
Cefaclor	>18	R	R
Cefotetan	>16	R	R
Cefoxitin	>18	R	R
Ceftriaxone	>21	R	R
Cephaloridene	>14	R	R
Cephalothin	>18	R	R
Chloramphenicol	>18	R	R
Chlortetracyline	>14	R	R
Clindamycin	>21	R	R
Cloxacillin	>9	R	R
Colistin	>11	R	R
Doxycycline	>16	R	R
Erythromycin	>23	R	R
Furazoldione	>14	R	R
Gentamicin	>15	R	R

Table 12 Antimicrobial Susceptibility Patterns (continued)

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Escherichia	Enterobacter
Imipenem	>16	R	R
Kanamycin	>18	R	R
Methicillin	>14	R	R
Mezocillin	>21	R	R
Nafcillin	>13	R	R
Nalidixic Acid	>19	R	R
Neomycin	>17	R	R
Nitrofurantoin	>14	R	R
Novobiocin	>22	R	R
Oxacillin	>20	R	R
Oxolinic Acid	>11	R	R
Oxytetracycline	>14	R	R
Penicillin	>19	R	R
Polymyxin B	>12	R	R
Rifampin	>20	R	R
Streptomycin	>15	R	R
Sulfamethizole	>10	R	R
Sulfamethoxazole with Trimethoprim	>10	S	S
Sulfathiazole	>10	S	S
Sulfsoxazole	>10	I	I
Tetracycline	>19	R	R
Tobramycin	>15	R	R
Timethoprim	>16	R	R

Table 12 Antimicrobial Susceptibility Patterns (continued)

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Escherichia	Enterobacter
Triple Sulfa	>10	S	S
Vancomycin	>12	R	R

R = Resistant

I = Intermediate

S = Susceptible

Table 13 Antimicrobial Susceptibility Patterns

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Serratia	Salmonella
Amdinocillin	>16	R	R
Amikacin	>17	R	R
Ampicillin	>17	R	R
Amoxicillin	>18	R	R
Bactracin	>13	R	R
Carbenicillin	>23	R	R
Cefaclor	>18	R	R
Cefotetan	>16	R	R
Cefoxitin	>18	R	R
Ceftriaxone	>21	R	R
Cephaloridеine	>14	R	R
Cephalothin	>18	R	R
Choramphenicol	>18	R	R
Chloretracycline	>14	R	R
Clindamycin	>21	R	R
Cloxacillin	>9	R	R
Colistin	>11	R	R
Doxycycline	>16	R	R
Erythromycin	>23	R	R
Furazolidone	>14	R	R
Gentamicin	>15	R	R
Imipenem	>16	R	R

Table 13 Antimicrobial Susceptibility Patterns (continued)

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Serratia	Salmonella
Kanamycin	>18	R	R
Methicillin	>14	R	R
Mezocillin	>21	R	R
Nafcillin	>13	R	R
Nalidixic Acid	>19	R	R
Neomycin	>17	R	R
Nitrofurantoin	>14	R	R
Nitrodurazone	>14	R	R
Novobiocin	>22	R	R
Oxacillin	>20	R	R
Oxolinic Acid	>11	R	R
Oxytetracycline	>14	R	R
Penicillin	>19	R	R
Polymyxin B	>12	R	R
Rifampin	>20	R	R
Streptomycin	>15	R	R
Sulfamethizole	>10	R	R
Sulfamethoxazole with Trimethoprim	>10	S	S
Sulfathiazole	>10	S	S
Sulfsoxazole	>10	I	I
Tetryacycline	>19	R	R
Tobramycin	>15	R	R

Table 13 Antimicrobial Susceptibility Patterns (continued)

Antimicrobial Agents	Zone of (in mm.) Susceptibility	Serratia	Salmonella
Trimethoprim	>16	R	R
Triple Sulfa	>10	S	S
Vancomycin	>12	R	R

R = Resistant

I = Intermediate

S = Susceptible

Table 14 Antimicrobial Susceptibility Patterns

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Klebsiella	Proteus
Amdinocillin	>16	R	R
Amikacin	>17	R	R
Ampicillin	>17	R	R
Bactracin	>13	R	R
Carbenicillin	>23	R	R
Cefaclor	>18	R	R
Cefotetan	>16	R	R
Cefoxitin	>18	R	R
Ceftriaxone	>21	R	R
Cephaloridine	>14	R	R
Cephalothin	>18	R	R
Choramphenicol	>18	R	R
Chlortetracycline	>14	R	R
Clindamycin	>21	R	R
Cloxacillin	>9	R	R
Colistin	>11	R	R
Doxycycline	>16	R	R
Erythromycin	>23	R	R
Furazolidone	>14	R	R
Gentamicin	>15	R	R
Imipenem	>16	R	R

Table 14 Antimicrobial Susceptibility Patterns (continued)

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Klebsiella	Proteus
Kanamycin	>18	R	R
Methicillin	>14	R	R
Mezocillin	>21	R	R
Nafcillin	>13	R	R
Nalidixic Acid	>19	R	R
Neomycin	>17	R	R
Nitrofurantoin	>14	R	R
Nitrofurazone	>14	R	R
Novobiocin	>22	R	R
Oxacillin	>20	R	R
Oxolinic Acid	>11	R	R
Oxytetracycline	>14	R	R
Penicillin	>19	R	R
Polymyxin B	>12	R	R
Rifampin	>20	R	R
Streptomycin	>15	R	R
Sulfamethizole	>10	R	R
Sulfamethoxazole with Trimethoprim	>10	S	S
Sulfathiazole	>10	S	S
Sulfsoxazole	>10	I	I
Tetracycline	>19	R	R

Table 14 Antimicrobial Susceptibility Patterns (continued)

Antimicrobial Agent	Zone of (in mm.) Susceptibility	Klebsiella	Proteus
Tobramycin	>15	R	R
Timethoprim	>16	R	R
Triple Sulfa	>10	S	S
Vancomycin	>12	R	R

R = resistant

I = Intermediate

S = Susceptible

DISCUSSION AND CONCLUSIONS

This investigation demonstrated that the New York Harbor area after rainfall, even moderate in volume has moderately high levels of coliform bacteria. These bacteria, which enter the harbor through the storm drain-sewer system and derived sewage treatment effluent, are resistant to a large spectrum of antimicrobial agents currently used in therapeutic situations.

This study confirms the results of a series of smaller, sporadic, one semester projects which monitored the presence of bacteria and their resistance at various sites in the harbor for 15 years (Corrigan, 1992). It appears that the resistance of the coliform bacteria is becoming a problem which is much greater in scope than previously considered.

Difficulty was encountered initially separating the various microbes from each sample site. The effective use of an array of differential and selective media provided an appropriate mechanism for isolating the various gram negative bacilli. Preliminary macromolecular testing provided a relatively inexpensive identification procedure, when compared to the significantly more rapid, reliable, and costly micromethod of the A.P.I 20E system. The differential agar analysis provided a mechanism for complete isolation and tentative identification. Future testing protocols will utilize this procedure.

Previous testing by St. Francis College students at several sites demonstrated the viability of coliform organisms at environmental temperatures as low as 5 C and in salinity concentrations as high as 40 ppt NaCl. Coliforms exhibiting these unusual physiological characteristics obtained them by plasmid transfer following genetic mutation. Basden (1982) studied biochemical variations and drug restivity of coliform isolated from the Gowanus Canal. Additional studies at the graduate level at Long Island University have attempted to determine if plasmid transfer is the mechanism used to develop resistance (Santore, 1992).

Antibiotic resistant coliforms are ubiquitous in New York Harbor year round. Their numbers increase dramatically with warm weather. Periodically, during moderate or heavy precipitation, the bacterial numbers reach extremely high numbers.

Previous studies of isolates from the Gowanus Canal collected in 1986 and used in a master's dissertation at Long Island University demonstrated a smaller spectrum of resistance for Escherichia, Klebsiella and Proteus (Giglio, 1990). While that study tested a smaller selection of antimicrobial agents, only 18 nearly half of the test agents were effective and sulfur drugs consistently demonstrated overall susceptibility.

New York Harbor is used for a wide variety of human activities all year. These activities include fishing, shellfishing and various recreational pursuits. During the warmer months of the year, beach areas provide an outlet for the large population of the city. The presence of large numbers of antibiotic resistant bacteria in the waters of the harbor can produce a health risk for the city's population. The development of diseases associated with the antibiotic resistant bacteria could establish a serious public health problem, especially with a greater number of immunodeficient individuals in that population. This would add an additional economic burden and high human cost to a severely strained city health care system. Finally, through tides and currents, the coliforms could move further up the Hudson River and along the coast to produce a problem of greater geographical scope.

RECOMMENDATION

Regular monitoring of key sites of coliform pollution is recommended to determine the level of antimicrobial resistance of these organisms. A cooperative monitoring program could be established by some of the metropolitan colleges and universities to conduct studies of this type on a limited basis. The data could then be integrated by the Hudson River Foundation. This procedure would help to minimize costs, stimulate undergraduate and graduate research projects on the harbor area, and provide an environmental interlocking mechanism between the Hudson River Foundation and institutions of higher education in New York City. Serological testing is also critical for final identification of coliform bacteria.

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REFERENCES

Basden, A. 1982. The biochemical variations and drug resistivity of coliform isolates from the Gowanus Canal. Master's Thesis. Long Island University, Brooklyn, New York.

Bauer, A.W., W.M.M. Kirgy, J.C. Sherris and M. Tuck. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45:493-496.

Cohen, M. 1992. Epidemiology of drug resistance: Implication for a post-antimicrobial era. Science 257:1050-1055.

Corrigan, J.E. 1992. Unpublished research.

Davies, J.E. and R. Rownd. 1972. Transmissible multiple drug resistance in Enterobacteriaceae. Science 176:758-768.

Dixon, B. 1986. Overdosing on wonder drugs. Science 201-40-43.

Ewing, W.H. 1986. Edwards and Ewing's Identification of Enterobacteriaceae Elseivier, New York.

Farmer, J.J.III, B.R. Davis and F.W. Hiskman-Brenner. 1985. Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. Journal of Clinical Microbiology 21:46-86.

Garrod, L.P. and F. O'Grady. 1973. Antibiotics and chemotherapy 4th edition. Churchill-Livingstone, Edinburg and London.

Giglio, P. 1990. Antibiotic resistance in Enterobacteriaceae isolated from the local waters of New York City. Master's Thesis. Long Island University, Brooklyn, New York.

Godwin, D. and J.H. Slater. 1979. The influence of growth environment on the stability of a drug resistant plasmid in Escherichia coli K12. Journal of General Microbiology 111:201-210.

Holt, J.G. (Editor in chief). 1984. Bergey's manual of systemic bacteriology. Volume I. Williams and Wilkins, Baltimore.

Kelch, W.J. And J.S. Lee. 1978. Antibiotic resistance patterns of gram negative bacteria isolated from envrionmental sources. Journal of Applied and Environmental Microbiology 36:450-456.

Krause, R.M. 1992. The Origin of Plagues: old and new. Science 257:1073-1078.

Kuntz, I.D. 1992. Structure based strategies for drug design and discovery. Science 257:1078-1082.

Murray, G.E., R.S. Tobin, B. Junkins and D.J. Kushner. 1984. Effect of chlorination on antibiotic resistance profiles of sewage related bacteria. Applied and Environmental Microbiology 48:73-77.

Neu, H.C. 1992. The crisis in antibiotic resistance. Science 257:1054-1072.

Richmond, M. 1983. Antibiotic resistance and the evaluation of bacteria. Nature 302:657-665.

Santore, T.A. 1992. Restriction endonuclease analysis of DNA from two isolates from the Gowanus Canal. Master's Thesis. Long Island University, Brooklyn, New York.

Difco Manual 1985. 10th edition. Difco Laboratories, Detroit, Michigan.