BACTERIOLOGIC STUDY OF A COMMUNITY WATER SUPPLY: DETECTION OF ENTEROTOXIGENIC ACTIVITY AND RESISTANCE TO ANTIMICROBIAL DRUGS[1]

Rafael García González,[2] María Lucía Taylor,[3] and Guillermo Alfaro[4]

A community water supply survey in Tlaxcala, Mexico, registered coliform counts considerably above the minimum WHO standards for acceptable drinking water. Subsequent testing of E. coli *and* Shigella *strains isolated during the survey showed extensive multiple plasmid-mediated resistance to common antibiotics and demonstrated the presence of enterotoxigenic strains of* E. coli.

Introduction

Mexico, like most developing and some developed countries, faces increasingly widespread water supply problems as a result of environmental pollution aggravated by rapid population growth and industrial development. Such pollution is tending to modify the physical, chemical, and biological equilibriums found in natural water supplies to a greater or lesser extent, impairing the water's quality and making it unfit for certain uses *(1,2)*.

One kind of environmental pollution that has an important effect on surface waters is bacterial contamination. In theory, many microbial pathogens could enter such water and use it as a medium for dissemination; but in fact those that do so are invariably bacteria from feces or (less commonly) urine from humans or animals that are sick or else are healthy carriers *(3)*. The introduction of such pathogens into water increases the chances of disease transmission and at times has led to epidemics or even pandemics of such waterborne diseases as bacillary dysentery, gastroenteritis, cholera, typhoid, paratyphoid, and others.

For these reasons, a community water supply's pollution index is generally viewed as providing a representative parameter for determining the relative predominance of certain pathogenic microorganisms and for assessing their potential adverse health impact—an impact manifested primarily in cases of diarrheal disease.

The problem is further complicated by the presence of antibiotic-resistant bacteria in nature. In this vein, plasmids bearing genes that confer resistance to various antibiotics are commonly found in bacteria isolated from the intestinal tract and from human and livestock feces *(4)*, and such bacteria in turn have been cited as contaminants of river and ocean waters, especially at sites near sewage outfalls *(5,6)*. It is therefore not surprising that the existence of resistant bacteria in the environment, which has been reported often in recent years, should pose a major problem for those

[1]Also appearing in Spanish in the *Boletín de la Oficina Sanitaria Panamericana* 93(2), 1982.

[2]Researcher, Microbiology Laboratory, Department of Human Ecology, School of Medicine, National Autonomous University of Mexico, Mexico City, Mexico.

[3]Researcher, Immunomicrobiology Laboratory, Department of Human Ecology, School of Medicine, National Autonomous University of Mexico.

[4]Researcher, Department of Biotechnology, Institute of Biomedical Research, National Autonomous University of Mexico.

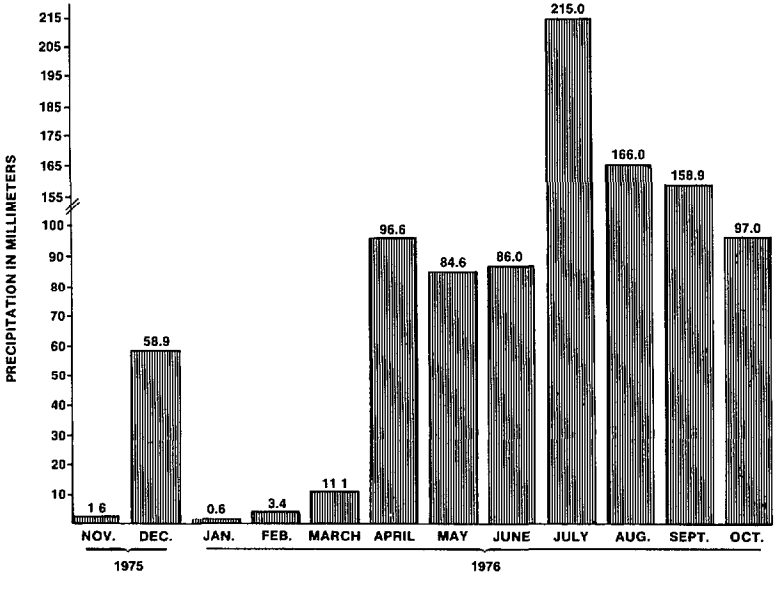
concerned with control of potential epidemics.

It is also true, however, that knowing the magnitude of these problems could pave the way for planning their reduction or elimination through gasteroenteric disease eradication projects. The investigation described, conducted with this in mind, was designed to perform two tasks: (1) bacteriologic analysis of a community water supply by methods permitting isolation of the coliform bacteria considered indicators of fecal contamination; and (2) analysis of the extrachromosomal genetic material of isolated bacteria for the purpose of correlating the presence and environmental distribution of particular plasmids with observed patterns of antibiotic resistance and enterotoxigenic activity.

The Setting

The *Tierra y Libertad* collective farming community, where the study was conducted, is located in Tlaxco, a rural municipality in the state of Tlaxcala. The region has rivers with numerous tributaries arising along the southern slopes of the Tlaxco Mountains. Despite this circumstance, however, about half the region's territory consists of unused eroded land, while the rest is devoted to seasonal farming. The local rainfall, as indicated in Figure 1, reached its highest recent levels in December 1975 and August-October 1976 *(7)*. The highway system provides good connections via paved roads with neighboring states.

Figure 1. Monthly rainfall in the *Tierra y Libertad* area in millimeters from November 1975 through October 1976. Source: Dirección General de Geografía y Meteorología *(7)*.



During the time of our study (November 1975-October 1976) the community of *Tierra y Libertad* was found to have 663 inhabitants, of whom 137 were economically active and engaged in the collective farming of oats on 700 hectares of land; 150 hectares of the communal lands were devoted to individual homes and habitations.

As of November 1975 the region's health and sanitary conditions were poor, there being a lack of medical care, hospital facilities, drainage systems, latrines, and water treatment. Overall annual mortality (10.6 deaths per 1,000 inhabitants) was high, as was infant mortality (92.9 deaths per 1,000 live births, as compared to a rate of 68.3 per 1,000 nationwide—*8*).

For the purpose of determining the magnitude of the existing problem, medical consultations were provided weekly over a two-month period for the inhabitants of *Tierra y Libertad.* The results of those consultations indicated that gastroenteric diseases accounted for 24.7 per cent of the disease cases diagnosed.

Materials and Methods

Water Sampling and Isolation of Bacteria

Water samples were collected from water stored in homes and from water supply sites. The sampling locations, selected to conform with WHO requirements, included seven points along the water supply system and 10 in-house storage tanks, the latter being chosen at random. All the samples were collected and transported under conditions that minimized chances for alteration of the samples' bacterial populations *(9,10).*

As shown in Table 1, biochemical and serologic examination of these samples demonstrated that numerous representatives of the family Enterobacteriaceae were present. An effort was therefore made to focus attention on the three genera *(Escherichia, Salmonella,* and *Shigella)* including agents responsible for infectious gastroenteritis. Table 2 lists media used to isolate bacteria from the water samples and to identify and maintain the isolates. Isolation of coliform, *Salmonella,* and *Shigella* bacilli was attempted using the multiple-tube technique *(9,10,12,13),* the cultures being incubated at 37°C for 24 hours—or for 48 hours in cases where slow lactose fermentation was involved. The most probable number (MPN) of coliform bacilli per 100 ml of water was then determined in accordance with WHO standards *(1,9,14),* and biochemical identification of the isolated microorganisms was carried out *(15-18).*

Drug Sensitivity and Serologic Testing

To determine the isolates' sensitivity to antimicrobial drugs, 0.01 ml inoculums containing approximately 10,000 cells per ml were taken from cultures incubated overnight and added to Petri dishes containing Luria medium and one of the following antimicrobials at the concentrations indicated in Table 2: nalidixic acid, ampicillin, chloramphenicol, streptomycin, spectinomycin, kanamycin, rifampicin, and tetracycline. Minimal medium was used to test for sensitivity to sulfamethoxazole. The dishes were then incubated at 37°C for 12-18 hours, at which time the degree of bacterial growth inhibition was determined *(19).*

Plaque agglutination tests were performed for purposes of serologically identifying the isolated strains. The test for *E. coli* included Polyvalent I (anti-OK-B-26-119), Polyvalent II (anti-OK-B-124-128) and *alkalescens-dispar* antisera (Behringwerke), while the test for *Shigella spp.* included glycerinated antisera for *Shigella* groups A, B, C, and D (Becton Dickinson) *(15,20).* These tests employed dark-field slides with grids (Hyland Laboratory, Costa Mesa, California) *(10,14).* In the case of a negative response to 0 antigen (due to

Table 1. Bacterial strains isolated and identified in water samples obtained in November 1975-October 1976 and in August-September 1977.

Strains isolated (by tribe, genus, and species)	Year and month of water sample collection														
	1975		1976										1977		
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Aug	Sep	Total
Escherichiea:															
Escherichia coli	4	6	6	10	5	4	3	7	14	9	11	1	7	5	92
Shigella spp.	1	0	3	5	1	5	1	6	2	5	8	5	3	3	48
Edwardsielleae:															
Edwardsiella spp.	1	1	3	3	1	1	1	0	0	0	0	0	0	0	11
Salmonelleae:															
Citrobacter freundii	7	7	7	6	5	10	10	8	9	11	8	5	12	12	117
Citrobacter diversus	3	2	5	0	4	4	4	3	0	3	4	0	7	2	41
Klebsielleae:															
Klebsiella pneumoniae	2	0	2	0	1	2	0	1	2	2	1	3	1	3	20
Enterobacter cloacae	2	0	1	1	2	1	3	0	3	2	2	3	8	3	31
Enterobacter aerogenes	11	9	3	0	1	2	5	3	0	0	0	0	1	2	37
Enterobacter hafniae	6	7	17	10	13	13	17	6	6	5	9	8	4	7	128
Enterobacter agglomerans	18	16	20	20	20	20	18	17	17	16	18	18	18	17	253
Serratia liquefaciens	2	4	2	3	4	2	2	3	1	2	2	2	2	2	33
Serratia marcensens	0	0	0	0	0	1	0	0	1	0	0	7	3	2	14
Serratia rubidaea	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2
Proteeae:															
Proteus vulgaris	5	5	6	1	6	5	7	5	2	1	0	4	3	1	51
Proteus morgani	7	1	3	1	1	4	5	4	3	0	0	1	0	2	32
Proteus mirabilis	5	6	0	5	2	1	2	0	0	2	0	2	1	4	30
Proteus rettgeri	0	0	0	0	2	2	0	2	0	0	0	0	2	1	9
Providencia alcalifaciens	1	0	0	1	1	0	1	0	1	0	2	0	0	0	7
Providencia stuartii	0	0	0	0	0	1	1	0	0	0	0	0	1	0	3
Total	75	64	78	66	69	79	80	65	61	58	65	59	73	67	959

Table 2. Media used for isolation and identification of bacteria collected in water samples from *Tierra y Libertad*.

Type of media	Media used
Initial isolation media	Broth and MacConkey agar at various concentrations (Difco)
Confirmation test media	Endo (Difco) Eosin-methylene blue agar (Merck) Brilliant green agar with 2% bile (Difco) *Shigella-Salmonella* agar (Merck)
Enrichment media	Broth for Gram negative bacteria *(15)* Tetrathionate broth (Difco) with 20% iodo-iodate solution *(16)* Cystine-selinite media (Merck) Peptonized solution *(15)*
Biochemical identification media *(12, 13,16,17)*	Agar-Kligler (Merck) Christensen's agar-urea (Merck) with 40% urea solution *(16)* Nutrient gelatin (Merck) Mannitol-red phenol agar (Merck) Voges-Proskauer methyl red (Merck) SIM medium (Difco) Simmons-agar citrate (Merck)
Maintenance media	Nutrient agar (Difco) Nutrient broth with egg
Other media	Minimal medium (M9) (see reference *11*) with the following additives: lactose, 0.2% (Merck); l-amino acids, 20 μg/ml (Merck); thymin, 50 μg/ml (Merck); vitamin B_1, 1 μg/ml (Merck); Bacto Agar, 2% (Difco); and sulfamethoxazole, 12.5 μg/ml (La Roche) Luria medium *(11)* with the following additives: thymin, 20 μg/ml (Merck); Bacto Agar, 2% (Difco); and the following antimicrobial drugs: nalidixic acid, 40 μg/ml (Sigma); ampicillin, 50 μg/ml (Wyeth-Vales); chloramphenicol, 25 μg/ml (Merck); streptomycin, 100 μg/ml (Lakeside); spectinomycin, 20 μg/ml (Upjohn); kanamycin, 50 μg/ml (Bristol Myers of Mexico); rifampicin, 10 μg/ml (Lepetit of Mexico); and tetracycline, 25 μg/ml (Carlo Erba).

masking with K antigen), the bacterial suspension was boiled for 30 to 60 minutes *(15, 18,20)*.

Plasmid Extraction and Characterization

Plasmid DNA was extracted and characterized electrophoretically in agarose gels using the method described by Alfaro *(21)*. That is, the bacterial isolates involved were cultured in 10 ml of Luria broth and incubated at 37°C until a density of about 5 x 10^6 cells per ml was reached. The cultures were then washed with TE buffer[5] and resuspended in 0.25 ml of the same buffer. Lysis was carried out at room temperature by the successive addition of 0.05 ml of NaOH (1M) and 0.05 ml of 10 per cent sodium dodecyl sulfate. The lysed cells were then brought to pH 7 (by adding 0.05 ml of 0.2 M Tris and 1.8 M Tris-HCl), incubated for five hours at 50°C, and kept at -20°C for 12 hours. The samples were subsequently thawed at room temperature, and most of the chromosomal DNA and proteins were removed by low-speed centrifugation.

The resulting samples were subjected to electrophoresis with BA buffer.[6] The electrophoretic tests were performed with cylindrical gels containing 0.8 per cent (weight by volume) of agarose. In general, 0.04 ml of the sample was mixed with 0.01 ml of a glycerol solution saturated with bromophenyl blue, and the test was run at 40 volts for five hours at 25°C.

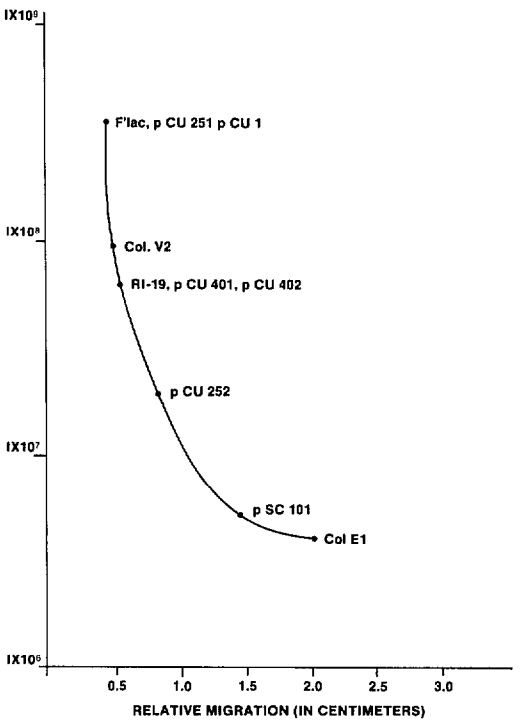
After electrophoresis was completed, the resulting DNA bands were stained with an aqueous solution (1 μg/ml) of ethidium bromide and visualized with a shortwave UV transilluminator (King FL8 BLB, Japan). Tri-X pan film (Kodak) and a red filter were used for taking photographs. The molecular weight of each plasmid was determined by measuring its electrophoretic mobility and plotting it upon a curve (of electrophoretic mobility versus the logarithm of molecular

[5]A buffer at pH 8.0 containing 0.01M Tris and 0.01M EDTA (ethylene diaminotetracetic tetrasodium salt).

[6]0.05M Tris, 0.02M sodium acetate, 0.018 sodium chloride, and 0.002M disodium EDTA.

weight) derived from prior electrophoretic testing of plasmids with known molecular weights under equivalent conditions (see Figure 2).

Figure 2. Relative electrophoretic migration of plasmids with different molecular weights. From M. López-López *(26)*, courtesy of the author.



Bacterial Conjugation

Conjugation was accomplished in the manner described by Alfaro and Willetts *(22,23)*. *E. coli* strain K-12 JM 1456 (without plasmids; requiring thymidine, tryptophan, and lactose; and resistant to nalidixic acid) *(24)* was used as the recipient strain, and *E. coli* isolates (from our water samples) exhibiting distinct patterns of resistance to antimicrobial drugs were used as donor strains.

Toxigenicity

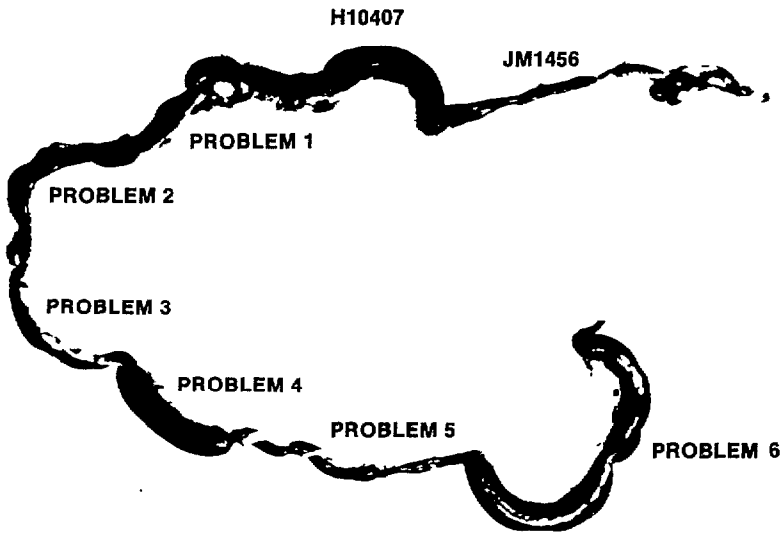
The isolates' potential toxigenicity was measured in male New Zealand rabbits weighing approximately 3 kg. These rabbits were maintained in the animal rooms of the National University Medical School on a diet of water *ad libitum* and purina. Each test animal was anesthetized with 3 mg per kg of sodium pentothal administered intravenously. The ligated small intestine segment test was then performed, followed by asepsis and antisepsis *(25)*, sterile filtrates of the bacterial test strains being inoculated separately into each 10 cm interval of intestine (see Photo 1). In addition, *E. coli* strain K-12 JM 1456 was inoculated as a negative control, and *E. coli* strain H-10407 CFA(+)[7] was inoculated as a positive control. Thereafter the abdomen was closed in stages, leaving the animal to rest at a temperature of 30°C for 18 hours. At the end of that period, before the death of the animal, the stitches were removed, again in stages, and the intestine was inspected to determine the length of each intestinal segment and the volume of material it contained.

Results

Analysis of the water samples collected in November 1975-October 1976 showed that coliform contamination was present in the community water supply from its points of origin to the community water system's intake sites. These findings indicated that the most probable number (MPN) of coliform bacilli at each of the supply sites sampled was in excess of three per 100 ml of water, the highest level considered acceptable by the World Health Organization *(9,10,14)*. Water stored in

[7]Supplied by the Medical Microbiology Laboratory of the National School of Biological Sciences, National Polytechnic Institute, Mexico.

Photo 1. Ligated segments of a rabbit intestine inoculated with *E. coli* control strains and isolates to test for enterotoxigenic activity. Strain K-12 JM 1456 served as the negative control and enterotoxigenic strain H-10407 CFA(+) served as the positive control. Segments 1, 2, 3, and 5 yielded negative results and segments 4 and 6 yielded positive results.



homes was also found to contain unacceptably high levels of coliform bacilli. A more detailed account of this survey and its findings has been presented elsewhere *(10)*.

As Table 3 shows, however, no salmonellae were detected in samples collected during this initial survey period. For this reason, a two-month survey of water samples (both inside and outside of homes) was conducted in August and September 1977, the months of heaviest rainfall that year; and a modified procedure was used for processing the samples. Again, despite detection of high MPN coliform levels (see Figure 3), no salmonellae were detected.

Drug Resistance

The aforementioned drug resistance tests showed that the *E. coli* and *Shigella* strains isolated were resistant to some of the drugs tested. Specifically, resistance to at least one drug was demonstrated by 92 *E. coli* isolates and 48 *Shigella* isolates. As Table 4 shows, the *E. coli* strains could be separated into 10 groups according to their patterns of drug resistance. The *Shigella* strains showed fewer distinct resistance patterns to the drugs tested, and their characterization thus required separation into fewer groups.

Plasmids

Bacterial isolates representative of the 10 resistant *E. coli* groups were selected for plasmid extraction and electrophoretic testing. As Photo 2 shows, this made it possible to detect strains carrying one or more extrachromosomal genetic elements. It should be noted, however, that the number of drugs resisted and the complexity of the resistance patterns did not show any clear correlation with the molecular weight of individual plasmids or with the number of different plasmids per cell (see Table 5). Nevertheless, it seems pertinent

Table 3. Serologic classification of *E. coli* and *Shigella* strains isolated from *Tierra y Libertad* water samples collected in November 1975-October 1976 and in August-September 1977, by month.

Month of sample collection	Samples collected from water sources								Samples collected from home water storage sites							
	No. of *E. coli* and *Shigella* isolates								No. of *E. coli* and *Shigella* isolates							
	E. coli serologic groups[a]			*Shigella* serologic groups[b]					*E. coli* serologic groups[a]			*Shigella* serologic groups[b]				
	I	II	*Alkalescens-dispar*	A	B	C	D	Total isolates	I	II	*Alkalescens-dispar*	A	B	C	D	Total isolates
Nov 75	2	2	0	0	0	0	1	5	0	0	0	0	0	0	0	0
Dec 75	2	2	0	0	0	0	0	4	1	1	0	0	0	0	0	2
Jan 76	1	1	0	0	0	0	1	3	2	2	0	2	0	0	0	6
Feb 76	3	1	0	0	0	0	4	8	4	2	0	0	0	0	1	7
Mar 76	1	1	1	0	0	0	1	4	1	1	0	0	0	0	0	2
Apr 76	2	0	0	0	1	0	0	3	2	0	0	3	1	0	0	6
May 76	0	1	0	0	0	0	1	2	0	2	0	0	0	0	0	2
Jun 76	1	1	1	0	0	0	3	6	2	2	0	0	1	0	2	7
Jul 76	4	3	0	0	0	0	1	8	1	4	2	0	0	0	1	8
Aug 76	1	4	0	0	0	0	4	9	2	2	0	0	0	0	1	5
Sep 76	2	4	0	0	3	0	2	11	1	4	0	1	0	2	0	8
Oct 76	1	0	0	0	0	0	3	4	0	0	0	1	0	0	1	2
Subtotal	20	20	2	0	4	0	21	67	16	20	2	7	2	2	6	55
Aug 77	1	2	1	0	0	0	6	10	1	2	0	0	0	0	1	4
Sep 77	0	3	0	0	0	0	6	9	1	1	0	0	0	0	0	2
Subtotal	1	5	1	0	0	0	12	19	2	3	0	0	0	0	1	6
Total	21	25	3	0	4	0	33	86	18	23	2	7	2	2	7	61

[a]Testing positivcly with polyvalent I (Anti-OK-B-26-119), polyvalent II (Anti-OK-B-124-128), or *alkalescens-dispar* antisera.
[b]Testing positively with either Group A *(S. dysenteriae),* Group B *(S. flexneri),* Group C *(S. boydii),* or Group D *(S. sonnei)* antisera.

Figure 3. The most probable number (MPN) of coliform bacteria in *Tierra y Libertad* water sources and home water storage sites per 100 ml of water, as determined on the basis of samples collected in August and September 1977. Each bar represents the MPN at a given water supply or home storage site. (Though the scale only goes up to an MPN of 1,100 per 100 ml, many of the results shown exceeded that number.)

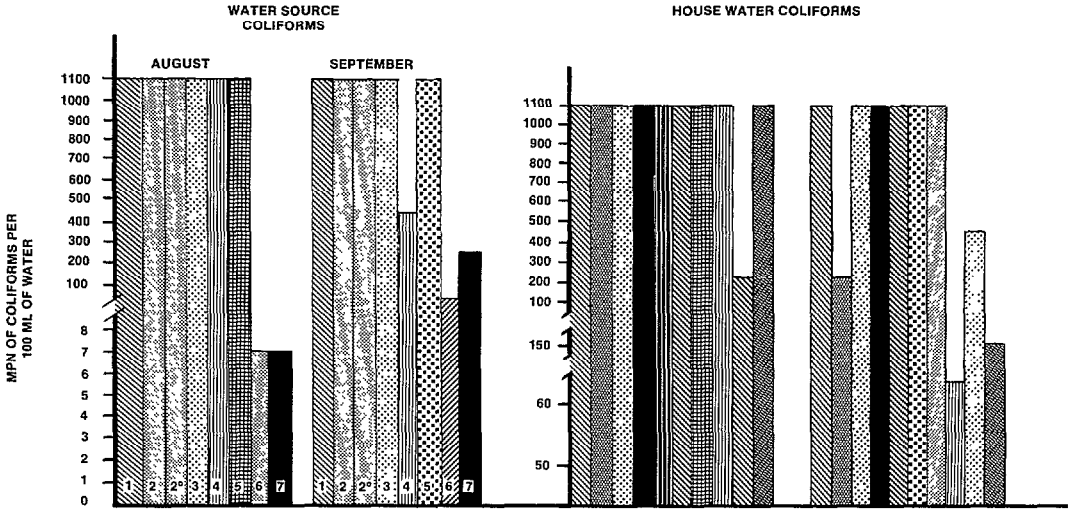
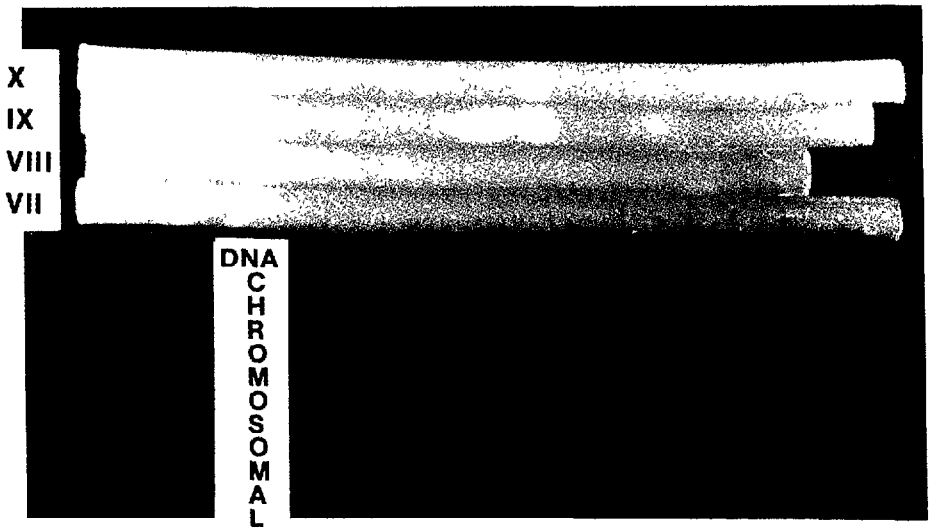


Table 4. Classification of *E. coli* and *Shigella spp.* isolates according to their antimicrobial drug resistance patterns.

Type of isolate	Drug resistance group	Test drugs resisted[a]	% of resistant strains in each group
Escherichia coli	I	Cm Sm Sp Su Tc Ap Km	41.66
	II	Cm Sm Sp Su Tc Ap	13.88
	III	Cm Tc Ap Km	8.33
	IV	Cm Tc Ap	8.33
	V	Ap	2.77
	VI	Km	5.55
	VII	Sm Tc Ap	2.77
	VIII	Tc Ap	5.55
	IX	Sp Tc	5.55
	X	Sp Tc Nal	5.55
Shigella spp.	I	Sp Rif Tc	20.38
	II	Sp Rif Tc Ap Km	4.16
	III	Sp Rif Tc Nal	2.05
	IV	Sm Sp Su Tc	68.75
	V	Sp Rif Tc Ap	2.08
	VI	Rif Tc	2.08

[a]Ap = Ampicillin; Cm = Chloramphenicol; Sm = Streptomycin; Sp = Spectinomycin; Km = Kanamycin; Nal = Nalidixic acid; Su = Sulfamethoxazole; Tc = Tetracycline; Rif = Rifampicin.

Photo 2. Electrophoretic migration through agarose gel of DNA in plasmids extracted from isolated *E. coli* strains. The results shown were obtained by testing strains belonging to group VII (resistant to ampicillin, streptomycin, and tetracycline), group VIII (resistant to ampicillin and tetracycline), group IX (resistant to spectinomycin and tetracycline), and group X (resistant to nalidixic acid, spectinomycin, and tetracycline).



that the resistance to ampicillin observed in group V strains appeared to arise from a plasmid with a molecular weight of 16 x 10^6 daltons, a weight similar to that of plasmids described by López in *Salmonella typhimurium* strains isolated from clinical samples *(26)*.

Plasmids detected in the other *E. coli* strains tested are still being investigated for the purpose of better defining the genetic information that they carry. We have not yet tested the isolated *Shigella* strains for plasmids, but it is certainly possible that various of the resistance markers detected will turn out to be carried on extrachromosomal material.

Conjugation

A key feature of plasmids is their potential for epidemic dissemination among one or more kinds of bacteria. For this reason, the identification of plasmids likely to be transmitted by conjugation is important.

In our study, all the resistant *E. coli* groups except VIII (which appeared to have no R-plasmids) were tested in conjugation experiments—the donor strains being representatives of the resistant groups and the recipient strain being *E. coli* K-12 JM 1456. Potential products of conjugation were grown in dishes of enriched media containing one of the various test antibiotics; donors were counterselected by adding nalidixic acid, the only test drug to which strain JM 1456 is resistant. Of the 10 resistant *E. coli* strains studied, only two (belonging to groups II and IV) were found to have transferred resistant plasmids

Table 5. Electrophoretic migration in centimeters and estimated molecular weight in daltons of plasmids extracted from *E. coli* strains manifesting different patterns of drug resistance.

Drug resistance group	Test drugs resisted	Migration of DNA bands in gel (in cm)	Estimated molecular weight of detected plasmids (in daltons)[a]
I	Cm Sm Sp Su Tc Ap Km	0.7200	31×10^6
II	Cm Sm Sp Su Tc Ap	0.6000	50×10^6
III	Cm Tc Ap Km	0.6000	50×10^6
		0.7200	31×10^6
		0.8928	23×10^6
		1.1200	9.1×10^6
IV	Cm Tc Ap	0.6000	50×10^6
		0.7200	31×10^6
		0.8928	23×10^6
V	Ap	0.8800	16×10^6
VI	Km	0.5500	62×10^6
VII	Sm Tc Ap	0.6818	35×10^6
VIII	Tc Ap	–	–
IX	Sp Tc	0.5000	92×10^6
		1.5900	4.7×10^6
		2.4090	–
		2.7272	–
X	Sp Tc Nal	0.6363	43×10^6
		2.7272	–

[a]These estimates were based on the experimental data shown and the chart from reference *26* displayed in Figure 2.

through conjugation (Table 6). In all cases the products of conjugation received the ability to resist three drugs (ampicillin, chloramphenicol, and tetracycline) from their donors.

Electrophoretic analysis of these transconjugants revealed a plasmid weighing 31×10^6 daltons, appearing similar in weight to ones detected in resistance groups III and IV (see Table 6). The similarities of the apparent molecular weights and most genetic markers of these group III and IV plasmids suggest that a group III strain could have lost its resistance to kanamycin and in this manner have given rise to group IV. Whether or not this is actually the case remains uncertain, however, and the limitations of our method do not permit measurement of small variations in the molecular weights of the respective plasmids.

Toxigenicity

The results of the ligated intestinal segment test showed that of the various *E. coli* strains isolated and tested, two of them (tested in the segments labeled "problem 4" and "problem 6" in Photo 1) exhibited enterotoxigenic activity. The control strains provided a good basis for assessing the enterotoxigenicity of the strains tested.

Discussion

Overall, the results of our one-year survey showed that *Tierra y Libertad's* drinking water never satisfied the WHO requirements for po-

Table 6. Products of conjugation tests employing *E. coli* from all drug-resistance groups except group VIII as donors and *E. coli* strain K-12 JM 1456[a] as the recipient.

Drug resistance group and resistance patterns of successfully conjugated *E. coli* donors		Recipient *E. coli* K-12 strain	Characteristics of transferred plasmids		
Group	Resistance pattern		Conferred drug resistance patterns	Electrophoretic migration (in cm)	Estimated molecular weight (in daltons)[b]
II	Cm Sm Sp Su Tc Ap	JM 1456	Cm Sp Tc Ap	0.7200	31×10^6
		"	Cm Tc Ap	0.7200	31×10^6
IV	Cm Tc Ap	"	Cm Sp Tc Ap	0.7200	31×10^6
		"	Cm Tc Ap	0.7200	31×10^6

[a]The recipient K-12 strain was plasmid-free, was resistant to nalidixic acid, and required Thy, Trp, and Lac.
[b]Estimates based on results shown and Figure 2 data *(26)*.

tability, in that the most probable number of coliforms always exceeded the maximum acceptable level of 3-10 bacteria per 100 ml of potable water—both in the springs supplying the water and in the water stored at homes *(9)* (see Figure 3).

Both sets of samples demonstrated a direct correlation between the most probable number of coliforms and rainfall, presumably as a result of human and animal wastes being carried into the water supplies. In this regard, it is noteworthy that the area through which the water passes to reach the community includes sites commonly used for grazing cattle. In general, the results obtained were in the range that would normally be expected, considering the poor state of the distribution and storage systems and the lack of water treatment.

Nearly all genera of the *Enterobacteriaceae* were detected in the water, including strains of enterotoxigenic *E. coli* and *Shigella,* two bacterial types that together are considered responsible for a large share of childhood gastroenteritis morbidity *(8)*. The failure to detect microbes of the genus *Salmonella,* despite special efforts to do so, could be due to the survey design as well as the time period of the second survey, since it is to be expected that salmonellae will generally be present in polluted water.

The presence of *Shigella* and *E. coli* pathogens in the water samples suggests that the water is contaminated with fecal matter and also that the isolated pathogens could be responsible for some of the gastroenteritis problems found among the inhabitants. These observations are very important, since many of the *Shigella* and *E. coli* pathogens isolated were found resistant to the antimicrobial drugs most commonly used against them, including chloramphenicol and ampicillin (see Table 4). The results, therefore, demonstrate a clear need for more restricted use of the drugs of choice with the aim of avoiding problems in treating disease cases.

Investigation of the drug-resistant *E. coli* isolates led to identification of various plasmids carrying genes for resistance to a variety of drugs. (Strains included in group VIII on the basis of their resistance patterns were not found to contain any R-plasmids, indicating that the resistance genes involved were carried on the bacterial chromosome.) Although it was not always possible to establish a correlation between the different plasmids' molecular weights and the various resistance patterns observed, the data obtained are expected to make a worthwhile contribution to later studies focusing upon the ecology of bacterial plasmids in Mexico.

The fact that the conjugation experiments revealed only four products of conjugation could be due to any of several reasons. Some of the R-plasmids may have possessed no

transfer mechanism, or the mechanism may have been ineffective under the experimental conditions used, or the recipient strain could have proved inappropriate for expression of the plasmids' genetic markers. Each of these circumstances could have substantially reduced the number of transconjugants detected.

The ligated intestinal segment test was performed to identify enterotoxigenic *E. coli* isolates. Such enterotoxigenicity, in the case of *E. coli,* is known to be plasmid-coded. Therefore, the positive results of the test provided direct confirmation of the existence of plasmids carrying genes for enterotoxigenicity.

Conclusions

Overall, the data obtained from this study lead to the following conclusions:

1) The coliform pollution index of *Tierra y Libertad's* drinking water exceeds the acceptable limits for potable water established by WHO.

2) Rainfall levels and lack of water treatment have contributed to this pollution; the presence of *Shigella spp., E. coli,* and other coliforms indicates fecal contamination.

3) In all, 52.8 per cent of the *E. coli* isolates and 96.2 per cent of the *Shigella* isolates were found resistant to one or more antimicrobial drugs in common use.

4) Conjugation experiments demonstrated transfer of R-plasmids, and a correlation was observed between the plasmids' molecular weights and the drug-resistant properties transferred.

5) Ligated intestinal segment tests demonstrated enterotoxigenicity in two groups of *E. coli* strains.

6) Without underestimating the importance of other factors involved in gastroenteric disease syndromes, one can conclude that drinking-water contamination in *Tierra y Libertad* is playing an important role as a cause of gastroenteritis by promoting transmission of potentially pathogenic microorganisms. The situation is not improved by the fact that many of the strains involved, in their natural state, carry genes for enterotoxigenicity or resistance to common drugs. This circumstance, combined with the pathogens' ability to transfer such genes to other bacteria lacking them, increases the threat of possible epidemics.

SUMMARY

A prolonged investigation of a community water supply was conducted in Tlaxcala, Mexico, for the purpose of gauging levels of bacterial contamination, isolating and identifying the bacteria involved, and determining the pathogenic potential of certain strains.

The settlement surveyed, a communal farm named *Tierra y Libertad,* had a population of some 663 inhabitants and was dedicated principally to raising oats. Health conditions in *Tierra y Libertad* were poor, and free medical consultations provided during the investigation indicated that gastroenteritis accounted for a major share of local disease cases. Water samples were obtained at seven points along the water supply system and from 10 water storage tanks in local homes during the period November 1975 through October 1976 and again in August and September of 1977.

Bacteria isolated from these samples included *E. coli, Shigella spp.,* and representatives of nearly every genus of the Enterobacteriaceae. Test results indicated that the most probable number of coliforms per 100 ml of water sample exceeded World Health Organization standards for minimally acceptable drinking water in virtually every case.

Further analysis provided considerably more information about potential pathogens isolated from the samples. Drug sensitivity testing of *E. coli* and *Shigella* isolates demonstrated resistance by 92 *E. coli* isolates and 48 *Shigella* isolates to one or more

common antibiotics. Extraction and electrophoretic testing of plasmids from strains exhibiting different patterns of drug resistance revealed the presence of various extrachromosomal genetic elements, though no direct correlation emerged between the apparent size of particular plasmids and the complexity of the multiple drug resistance patterns involved. *E. coli* conjugation experiments demonstrated the transfer of R-plasmids in a limited number of specific cases. And tests performed with ligated rabbit intestines demonstrated that some of the *E. coli* isolates had enterotoxigenic potential.

Overall, these findings strongly suggest that drinking-water contamination is promoting gastroenteritis in *Tierra y Libertad* by facilitating transmission of potential pathogens. The situation is aggravated by the fact that many of the organisms studied carry genes for enterotoxigenicity or resistance to one or more antibiotics.

REFERENCES

(1) Guinea, J., J. Sancho, and R. Pares. *Análisis microbiológico de aguas: Aspectos aplicados.* Ediciones Omega, Barcelona, 1979, pp. 1-112.

(2) Strobbe, M. A. *Orígenes y control de la contaminación ambiental.* Editorial Continental, Mexico City, 1977, pp. 30-51.

(3) Pérez-Miravete, A. Fuentes de infección y transmisión de Salmonelosis. *Revista de Salud Pública Mexicana* 16:37-48, 1974.

(4) Williams-Smith, R., and M. A. Lingood. The transmissible nature of enterotoxin production in a human enteropathogenic strain of *Escherichia coli. J Med Microbiol* 4:301-307, 1971.

(5) Linton, K. B., M. H. Richmond, R. Bevan, and W. D. Guillespie. Antibiotic resistance and R factor in coliform bacilli isolated from hospital and domestic sewage. *J Med Microbiol* 7:91-100, 1974.

(6) Williams-Smith, S. Incidence in river water of *Escherichia coli* containing R factor. *Nature* 228: 1286-1288, 1970.

(7) Dirección General de Geografía y Meteorología. *Servicio Meteorológico Nacional: Boletín Climatológico.* Mexico City, 1970-1976.

(8) Dirección de Bioestadística. Estadísticas vitales de los Estados Unidos Mexicanos. Mexico City, 1971-1975.

(9) World Health Organization. *International Standards for Drinking-Water (Third edition).* Geneva, 1971.

(10) García-González, R., and M. L. Taylor. Estudio bacteriológico del agua de consumo de una comunidad. *Revista de Salud Pública Mexicana* 19: 715-727, 1977.

(11) Clowes, R. C., and W. Hayes (eds.). *Experiments in Microbial Genetics.* Blackwell Scientific Publications, 1968, p. 187.

(12) Pawsey, R. K. *Techniques with Bacteria: A Guidebook for Teachers.* Hutchinson Educational, London, 1974, pp. 122-159.

(13) Bodily, H. L., E. L. Updike, and J. O. Mason (eds.). *Diagnostic Procedures.* American Public Health Association, New York, 1970, pp. 227-280.

(14) Dalla-Valle, J. M. Note on the "most probable number" index as used in bacteriology. *Public Health Rep* 56:229-231, 1941.

(15) Edwards, P. R., and W. H. Ewings. *Identification of Enterobacteriacea (Third edition).* Burgess, Minneapolis, 1974, pp. 1-337.

(16) Branson, D. *Methods in Clinical Bacteriology: A Manual of Tests and Procedures.* C. T. Charles; St. Joseph, Missouri, 1972, pp. 10-49.

(17) MacFaddin, J. F. *Biochemical Tests for Identification of Medical Bacteria.* Williams and Wilkins, Baltimore, 1976.

(18) Bailey, W. P., and E. Scott. *Diagnostic Microbiology (Fourth edition).* C. V. Mosby, St. Louis, 1974, pp. 135-162.

(19) Alfaro, G., J. Martuscelli, and P. Mendoza-Hernández. Antibiotic resistance and phage-types of *Salmonella typhi* strains isolated in Mexico City. *Rev Lat Am Microbiol* 20:5-11, 1978.

(20) Bryant, N. T. *Laboratory Immunology and Serology.* W. B. Saunders, Philadelphia, 1979, pp. 83-95.

(21) López-López, M., and G. Alfaro. Resistencia a antibióticos en cepas de *S. typhimurium* aisladas de muestras clínicas: Caracterización de algunos plasmidos. *Rev Lat Am Microbiol* (in press).

(22) Miller, J. H. *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory, 1972, pp. 82-107.

(23) Alfaro, G., and N. S. Willets. The relationship between the transfer systems of some bacterial plasmids. *Genet Rev* 20:279-289, 1972.

(24) Alfaro, G. Related R plasmids in *Salmonella*

typhi strains isolated in Mexico City from clinical samples. *Rev Lat Am Microbiol* 19:209-215, 1977.

(25) Smith, H. W., and C. L. Gyles. The effect of cell-free fluids prepared from cultures of human and animal enteropathogenic strains of *Escherichia coli* on ligated intestinal segments of rabbits and pigs. *J Med Microbiol* 3:403-409, 1970.

(26) López-López, M. Análisis genético de plásmidos detectados en enterobacterias. Thesis, School of Chemistry, National Autonomous University of Mexico, Mexico City, 1977.

EDITORIAL PROFILE
MISS ELSIE MORCOM RETIRES

Miss Elsie J. Morcom, Chief of PAHO's Office of Publications, retired on 30 November 1982 after serving the Organization for 29 years. Miss Morcom, who came to supplement her exceptional language abilities with editing, publishing, and management skills, began working for PAHO in 1948. After leaving the Organization temporarily in 1953 and working briefly for other international agencies (the Pan American Institute of Geography and History and the Food and Agriculture Organization of the United Nations), she returned to PAHO, first as a translator and then as an editor, in 1958.

Born in Monterrey, Mexico, and educated in the United States, Miss Morcom possesses extraordinary command of both Spanish and English, in addition to her working knowledge of French and Portuguese.

Over the years Miss Morcom assumed increasing editorial responsibility for the *Annual Report of the Director,* which is published in both Spanish and English, and for a broad range of PAHO scientific publications. She became Chief of the Office of Publications in 1981. As Dr. Manuel Bobenrieth, Chief of the Office of Health and Biomedical Publications, observed at a farewell party for Miss Morcom last November, "She has exercised fine judgment in performing her assigned tasks, and has given us grounds to expect conscientious work of the highest quality; she has shown other professional and general services personnel consideration, respect, firmness, and support; she has used discretion while providing excellent collaboration; and...she has dedicated her life to the noble cause of health as it is served by publications that disseminate information capable of reducing suffering and saving lives."