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Escherichia coli in Settled-Dust and Air Samples Collected in Residential Environments in Mexico City

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***Escherichia coli*, an important indicator of the presence of fecal material, was isolated from indoor and outdoor environments in Mexico City. The heterogeneity of *E. coli* was represented by 89 serotypes, most of them coming from settled-dust indoor samples; 21% of them presented antibiotic multiresistance. The numbers of plasmids were higher among the antibiotic-resistant strains. The results of this study suggest that intestinal infections produced by environmental strains could be of more epidemiological impact than previously thought.**

The water and soil in Mexico City are believed to be contaminated with human waste, in view of the fact that waste treatment plants and the public waste disposal service are insufficient for 18 million inhabitants (7). In Mexico, the number of diarrheal episodes among children less than 6 years old has been associated with *Escherichia coli* (4, 5, 21).

The main purpose of this study was to determine the occurrence of *E. coli* in both airborne and settled dust collected from indoor and outdoor environments. Moreover, the serotypes, antibiotic resistance profiles, and presence of extrachromosomal DNA (plasmids) were analyzed.

Airborne and settled dust were sampled at 10 a.m. under undisturbed conditions from the living rooms of 30 homes located in the southern part of Mexico City. Airborne bacteria were collected with a two-stage Andersen sampler (Graseby Andersen, Atlanta, Ga.), with a constant air flow rate of 28 liters min^{-1} for 15 min (0.42 m^3 of air sampled). Samplers were loaded with petri dishes containing Trypticase soy agar (Difco Laboratories, Detroit, Mich.), amended with cycloheximide (50 $\mu g\ ml^{-1}$) to inhibit fungal growth and 5 mM glycine betaine (Sigma Chemical Co., St. Louis, Mo.) as an osmoprotector.

Settled-dust samples were collected with a vaccum cleaner (VK 121; Vorwerk, Madrid, Spain) in sterile bags; 2 m^2 was sampled during 4 min, from either carpet or flooring and from the patio cement.

Suspensions from settled dust were prepared by vortexing of 100 mg of sieved (149-μm-pore-diameter) settled-dust samples in 10 ml of dilution water (0.01 Tween 80, 1% bacteriological peptone, 2% inositol) for 2 min. Duplicate 200-μl aliquots of the suspension and 10-fold dilutions were spread on Trypticase soy agar plates (Difco) containing cycloheximide at 50 $\mu g\ ml^{-1}$ and onto violet-red-bile-glucose-agar plates (VRBG; Oxoid, Hampshire, England). Fecal coliforms were evaluated directly from dust suspensions in EC broth (BBL, Cockeysville, Md.) by the multiple-tube fermentation technique. Bacterial growth was determined by plating of positive tubes on VRBG.

All plates were incubated at 35°C for 24 to 48 h. The gram-negative bacteria were identified according to standard biochemical tests for enteric bacteria (API 20-E; bioMerieux Vitek, Inc., Hazelwood, Mo.). The identities of the *E. coli* strains isolated were confirmed by different biochemical tests (14).

The antimicrobial susceptibility of isolated *E. coli* strains was evaluated with the UniScept KB gram-negative type 2 test (bioMerieux Vitek, Inc.). Rabbit antisera (SERUNAM; Mexico) against 175 somatic (O) and 56 flagellar (H) antigens were used to determine the serotypes of the *E. coli* strains (16).

Plasmid DNA was extracted by the alkaline lysis procedure described by Birnboim and Doly (2); it was separated by electrophoresis on a 0.8% agarose gel in 1% Tris-borate-EDTA buffer containing 0.5 μg of ethidium bromide ml^{-1} at 110 V for 2 h at room temperature. The DNA was subsequently visualized with a shortwave UV light transilluminator (Macro) and photographed with Polaroid type 667 film.

The amount of airborne gram-negative bacteria from indoors represented 2.7% of the culturable airborne bacteria, while that from the outdoors represented 1.2%. From settled-dust samples, the proportions of gram-negative bacteria with respect to the total bacterial counts were 22.9% for indoors and 9.6% for outdoors. The frequency of isolation of *E. coli* from settled dust was 41.1% with respect to total coliform bacteria according to the multiple-tube fermentation technique.

E. coli serotypes isolated from dust samples are shown in Table 1. A high heterogeneity (70 serotypes) was obtained from settled-dust samples collected indoors, while 5 serotypes were obtained from air samples. The serotypes O20:H^-, O28:H^-, O86:H34, O127:H9, and O142:H34 isolated in this study have been associated with pathogenic types of *E. coli.*

Fourteen percent of the *E. coli* strains isolated showed susceptibility to more than two antimicrobials (Table 2); most of them presented resistance to ampicillin, ticarcillin, piperacillin, and tetracycline. Most of these isolates came from indoor settled dust, while only two antibiotic-resistant strains were from air samples.

The plasmid analysis of antibiotic-sensitive strains showed one or two plasmids (Fig. 1A), mainly of low molecular size, while antibiotic-resistant strains presented from two to five plasmids (Fig. 1B and Table 3).

In the present study, *E. coli* was isolated from among the airborne gram-negative bacteria, even though the isolation of this bacterium has previously only been reported in environments in which the main source of contamination should be human or animal fecal material, such as domestic waste treatment facilities (6, 17).

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TABLE 1. Serotypes of *E. coli* isolated from dust samples

Serotype	Serotype (relative frequency of isolation)		
	Indoor airborne dust	Settled dust	
		Outdoor	Indoor
Most common	O?:H1 (1) O8:H1 (1) O70:H11 (1) O157:H49 (1)	O8:H19 (5) O40:H30 (5) O51:H2 (4) O87:H21 (6)	O?:H$^-$ (7) O8:H51 (5) O39:H11 (11) O58:H30 (18) O70:H11 (13) O79:H40 (5) O86:H10 (18) O91:H10 (10) O166:H21 (5) O170:H$^-$ (5)
Pathogenic	O86:H34 (1)		O20:H$^-$ (1) O28:H$^-$ (2) O86:H34 (3) O127:H9 (1) O142:H34 (3)

The proportion of gram-negative bacteria from indoor settled dust was 22.9%, twice that found in outdoor environments. Also, the frequency of indoor isolation of *E. coli* was up to 33%, suggesting that this bacterium could be protected in indoor environments. *E. coli* was isolated from airborne dust at a low frequency; it could be associated with either the undisturbed samplings or with the presence of viable nonculturable strains. Interestingly, the isolation was possible when glycine betaine was used as an osmoprotector (13), suggesting that *E. coli* might be damaged during the aerosolization process (3).

TABLE 2. Antibiotic-resistant *E. coli* strains isolated from settled- and airborne-dust samples collected from indoor environments

Serotype	Antibiotic resistance[a]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Indoor soil																		
OR:H30		R				R	R	R										
O?:H$^-$					R	R	R											R
O?:H10	R				R	R	R											R
O6:H16		R						R				R					R	R
O7:H30	R				R	R	R											R
O8:H2					R	R	R											R
O8:H30					R	R	R								R	R	R	R
O11:H11					R	R	R											R
O20:H$^-$					R	R	R											R
O25:H30	R				R	R	R										R	R
O26:H30	R													R	R	R		R
O45:H28	R	R													R			R
O70:H11					R	R	R											R
O79:H40	R				R	R	R											R
O103:H21	R				R	R	R											R
O132:H7					R	R	R											R
O155:H4	R				R													R
O157:H32					R	R											R	R
O170:H$^-$					R	R	R										R	R
Indoor air																		
O70:H11					R	R	R											R
O157:H49	R				R	R	R	R							R	R	R	R

[a] R, resistant. Antibiotics are represented by numbers as follows: 1, trimethoprim; 2, nitrofurantoin; 3, norfloxacin; 4, ciprofloxacin; 5, ampicillin; 6, ticarcillin; 7, piperacillin; 8, cephalothin; 9, cefaxolin; 10, cefoxitin; 11, cefuroxime; 12, cefotaxime; 13, ceftazidime; 14, amikacin; 15, gentamicin; 16, tobramycin; 17, chloramphenicol; 18, tetracycline.

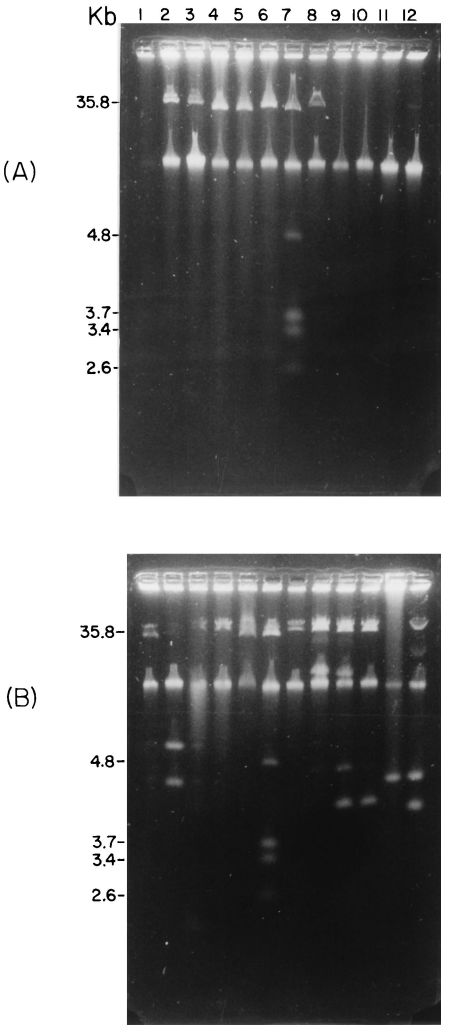

FIG. 1. Agarose gel electrophoresis of plasmids isolated from environmental strains of *E. coli*. (A) Lanes 1 to 6 and 8 to 12, antibiotic-sensitive strains; lane 7, molecular size plasmid standards from *E. coli* v517. (B) Lanes 1 to 5 and 7 to 12, antibiotic-resistant strains; lane 6, molecular size plasmid standards from *E. coli* v517.

Most of the *E. coli* serotypes obtained in this study belong to the indigenous flora of the human intestine; however, some are associated with the pathotypes related to diarrhea. Considering their somatic (O) and flagellar (H) antigens, *E. coli* has been classified into four groups: enteropathogenic (EPEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), and enterohemorrhagic (EHEC). On the other hand, on the basis of this classification, five pathogenic strains (O20:H$^-$, O28:H$^-$, O86:H34, O127:H9, and O142:H34) belonging to all of these groups except for EIEC were isolated from indoor settled dust (12).

One strain of the O157 serogroup was isolated; it is important because the EHEC group included as a prototype O157:H7, and it has been associated with different outbreaks and cases of hemorrhagic colitis and hemolytic-uremic syndrome (18).

The number of plasmid DNA bands was higher among the resistant *E. coli* strains than among the sensitive strains. Sev-

TABLE 3. Numbers and sizes of plasmid bands from *E. coli* strains resistant and sensitive to antimicrobials

Plasmid DNA band no. or size	No. (%) of plasmid bands in:	
	Resistant stains (n = 30)	Sensitive strains (n = 30)
Airborne dust		
Plasmid DNA band no.	3 (10)	2 (6)
1–2	1 (33)	2 (100)
3–5	1 (33)	0 (0)
>5	1 (33)	0 (0)
Mol size of band(s) (kb)[a]		
<2.6–20	8 (68)	3 (75)
21–38	1 (8)	1 (25)
38–56	1 (8)	0 (0)
>56	2 (16)	0 (0)
Settled dust		
Plasmid DNA band no.	21 (70)	17 (56)
1–2	9 (43)	15 (88)
3–5	10 (48)	2 (12)
>5	2 (9)	0 (0)
Mol size of band(s) (kb)[a]		
<2.6–20	45 (72)	7 (33)
21–38	9 (15)	7 (33)
38–56	0 (0)	1 (6)
>56	8 (13)	6 (28)

[a] Sizes were estimated by comparison with reference plasmids of *E. coli* v517 extracted and separated under the same conditions.

eral studies have focused on the association between plasmids and antibiotic resistance (10, 15). Moreover, some critical virulence properties can be encoded in plasmids, such as the EPEC enteroadherent factor plasmid, harboring the bundle-forming pili (9), and the ETEC plasmids, encoding the heat-labile or heat-stable enterotoxins or both (1).

It has been reported that different bacteria present an association between antibiotic and environmental resistance (8, 11). In the present study, 14% of the *E. coli* strains isolated showed multiresistance to antimicrobials, and most of them were isolated from settled dust in indoor environments. These strains were resistant to trimethroprim and tetracycline, among others, maybe due to the wide consumption of these antimicrobials (19, 20). Among the multiresistant strains, some serogroups were identified that could belong to pathogenic *E. coli* groups, such as O8, O25, and O20 (ETEC); O26 (EPEC); and O157 (EHEC).

The potential risk associated with the ingestion of food or water contaminated with *E. coli* present in the environment is the widespread dissemination of strains involved in human diarrhea. Also, the natural conjugal transfer of plasmids between human and animal pathogenic and nonpathogenic bacteria could be fostered.

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