Effect of Antibiotics on the Prevalence of Enterotoxigenic 
*Escherichia coli* in Two Populations in the Philippines

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Hostesses and restaurant employees in the Philippines were studied to determine whether an increased use of antibiotics was associated with a higher point prevalence of enterotoxigenic *Escherichia coli*. Of 1,030 hostesses and 628 restaurant employees, 28 and 4%, respectively, said that they had taken antibiotics within a week of being cultured (*P* < 0.001). Of hostesses and restaurant employees, 10% (103 of 1,030) and 2% (14 of 628), respectively, had antibiotics detectable in their urine (*P* < 0.001). Enterotoxigenic *E. coli* strains were isolated from 1.2% (12 of 1,030) of hostesses and 1.7% (11 of 628) of restaurant employees. In both populations, enterotoxigenic *E. coli* strains were never found in subjects who had antibacterial activity in their urine. Although resistance to two or more antibiotics was found more frequently in *E. coli* isolated from hostesses than in that isolated from restaurant workers (48 versus 33%; *P* < 0.01), antibiotic selective pressure did not increase the prevalence of enterotoxigenic *E. coli* in these two populations.

Enterotoxigenic (tox*) *Escherichia coli* is a common cause of diarrheal disease in developing tropical countries (7, 15, 17). Genes controlling the production of heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) are located on plasmids designated Ent (11, 21). In the Far East, these enteric pathogens are often resistant to multiple antibiotics; of 31 of these isolates, 80% transferred antibiotic resistance in bacterial mating experiments (8). In 35% of the matings transferring antibiotic resistance, the ability to produce enterotoxin was also transmitted to the recipients. Antibiotic selection increases the probability of detection of *in vitro* conjugation and provides for the selection of *R* plasmid-containing bacteria in the intestinal flora (1, 18). Smith and Linggood, among others, have suggested that antibiotics may also increase the prevalence of *E. coli* containing plasmids which contribute to enteropathogenicity (8, 10, 19–21).

A more direct effect of antibiotics on the selection of *Ent* has been proposed by several recent observations. In an experiment with an *E. coli* strain isolated from a pig with diarrhea, Gyles et al. (12) transduced via P1 coliphage both antibiotic resistance and enterotoxin production, implying that the genes controlling both traits were located on the same plasmid. Ampicillin resistance has also been found to transpose onto an *Ent* plasmid in an *E. coli* strain of human origin (13). If genes encoding for antibiotic resistance and enterotoxin production are frequently carried on the same plasmid, antibiotic selective pressure should increase the prevalence of tox*+* *E. coli* in the human gastrointestinal tract. To test these hypotheses in vivo, we compared the point prevalence of LT*+* ST*+* and LT*+* ST*+* *E. coli* in relation to antibiotic consumption in two populations in the Philippines.

MATERIALS AND METHODS

Study population. A total of 1,030 hostesses attending the social hygiene clinic for cervical cultures to detect *Neisseria gonorrhoeae* and 628 individuals working in restaurants in Angeles City, Republic of the Philippines, were cultured for enteric pathogens. Of the restaurant workers (RWs), 72% were women of the same age as the hostesses who lived and worked in the same areas of Angeles City. Specimens were collected within a 10-day period in May 1979. All of the hostesses and RWs had formed stools when the cultures were obtained.

Collection of specimens. Rectal cultures were inoculated into Cary-Blair transport medium, stored at 4°C, and processed within 2 weeks. A 1- or 2-ml amount of urine was frozen at −70°C and examined for antibiotic activity within 5 weeks of collection.

Processing of specimens. Specimens were cultured on MacConkey, Hecktoen, and thiosulfate citrate bile salts sucrose (TCBS) media and inoculated into Hajna broth and alkaline peptone water (APW), pH 8.5. The Hajna broth was subcultured on Hecktoen and deoxycholate media (Difco Laboratories, Detroit, Mich.) after overnight incubation at 37°C. A subculture of APW was taken after 6 h and cultured on TCBS medium. *Salmonella*, *Shigella*, and *Vibrio* species were identified by standard methods (5), employing the API 20E system, and with the use of commercial antisera (Difco).

Identification of tox*+* isolates. Ten lactose-positive colonies with an appearance typical of *E. coli*...
were selected from the MacConkey medium, stored on nutrient agar slant cultures, and tested within 2 months of isolation for the production of LT. Organisms that were LT+ were retested for LT and ST. No attempt was made to test for LT ST+ E. coli. Bacteria to be screened for LT were inoculated into 1 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 0.6% yeast extract (Difco) and incubated stationary at 35°C for 48 h at 37°C. Isolates tested for both LT production and ST production were inoculated into 5 ml of a similar medium and incubated in a tissue culture roller tube apparatus at 8 rpm at 37°C for 24 h. Supernatants were tested for LT and ST in Y1 adrenal cells (16) and sucking mouse assays simultaneously (6). All tox+ strains were identified with the API 20E system.

**Antibiotic susceptibility testing.** Antibiotic susceptibilities of all tox+ and five non-enterotoxigenic (tox-) E. coli strains isolated from each subject were determined by the agar dilution method with Mueller-Hinton agar and broth. The minimum inhibitory concentration for resistant organisms by agar dilution was >16 µg/ml for tetracycline, streptomycin, ampicillin, and kanamycin and >8 µg/ml for gentamicin and chloramphenicol. Antibiotic susceptibilities to neomycin (30 µg) and sulfisoxazole (250 µg) were determined by the agar diffusion method (3). Cultures (24 h) of the isolates were adjusted to an 85% transmission at 550 nm in a Coleman Jr. II spectrophotometer (equal to the turbidity of a 0.5 McFarland barium sulfate turbidity standard [2]). E. coli ATCC 25922, susceptible to all antibiotics, *Pseudomonas aeruginosa* ATCC 27858, resistant to ampicillin, chloramphenicol, kanamycin, and neomycin, and a clinical isolate from the Philippines, *E. coli* 203, resistant to ampicillin, chloramphenicol, gentamicin, kanamycin, neomycin, streptomycin, sulfisoxazole, and tetracycline, were included as controls when clinical isolates were tested for antimicrobial susceptibility.

**Urine testing for antibiotic activity.** *Bacillus subtilis* ATCC 6633, susceptible to all the tested antibiotics, was grown overnight in Mueller-Hinton broth, adjusted to an 85% transmission, and spread onto a Mueller-Hinton agar plate. A 10-µl amount of urine was placed onto the plate, which was incubated at 37°C for 24 h. Clear zones indicated the presence of antibiotics in the urine (4, 9).

To determine the sensitivity and specificity of the urine test, random urine specimens from laboratory personnel who had not received antibiotics in the previous 2 weeks were tested. Urine specimens were also collected from individuals 6, 24, and 48 h after they had ingested either 250 mg of penicillin, 250 mg of tetracycline, 250 mg of ampicillin, or 500 mg of sulfaguanidine.

**RESULTS**

**Specificity and sensitivity of urine tests.** None of 23 random urine specimens produced clear zones on Mueller-Hinton agar plates inoculated with *B. subtilis*. Antibiotic activity was detected in all urine specimens collected 6 and 24 h, but not 48 h, after the antibiotics had been ingested.

**Study populations.** Of 1,030 hostesses and 628 RWs, 28 and 4%, respectively, gave a history of taking antibiotics within a week of being cultured (*P < 0.001*) (chi square). Of hostesses and RWs, 10% (103 of 1,030) and 2% (14 of 628), respectively, had antibiotics detectable in their urine (*P < 0.001*). Of 103 hostesses with antibacterial activity in their urine, 65 (63%) denied prior ingestion of antibiotics, which implies that antibiotic use was greater than reported. Of the hostesses who admitted taking prior antibiotics, 80% received ampicillin or penicillin. Of 603 RWs from whom urine was available, 3 (<1%) denied previous therapy but had antimicrobial activity in their urine, suggesting that RWs were, in fact, taking antibiotics infrequently. RWs received a variety of different antibiotics, including ampicillin, chloramphenicol, neomycin, sulfaguanidine, and tetracycline.

There was no significant difference in the isolation rate of *Salmonella* sp., *Shigella*, sp., and *Vibrio parahaemolyticus* between hostesses and RWs (8 of 1,030 versus 7 of 628; *P > 0.50*). LT ST+ and LT+ ST- *E. coli* strains were isolated from 12 of 1,030 (1.2%) hostesses and from 11 of 628 (1.7%) RWs (*P > 0.40*). None of the individuals infected with this pathogen had antibiotics detectable in their urine. One hostess who said that she had recently received tetracycline was infected with a tetracycline-resistant LT+ ST- *E. coli* strain. One hostess and one RW were infected with LT+ ST+ *E. coli*. The remaining tox+ *E. coli* strains identified produced LT alone.

The percentages of antibiotic resistance among tox+ *E. coli* strains (five isolates per subject) isolated from cultures of 300 hostesses, selected in proportion to the total number cultured at the clinic on a specific day, and 136 random RWs (29 and 22% of the population, respectively) are listed in Table 1. Of tox+ *E. coli* strains isolated from hostesses and RWs, 48% (722 of 1,500) and 33% (226 of 680), respectively, were resistant to two or more antibiotics (*P < 0.01*). Of tox+ *E. coli* strains isolated from hostesses and RWs, 57% (28 of 49) and 32% (8 of 25), respectively, were resistant to two or more antibiotics (*P < 0.10*).

**DISCUSSION**

Resistance to two or more antibiotics was more frequent among *E. coli* strains isolated from hostesses than among those isolated from RWs, suggesting that antibiotics had selected for R plasmid-mediated resistance in one population in comparison with the other. The point prevalence of tox+ *E. coli* was similar among hostesses and RWs, indicating that in the Phil-
TABLE 1. Antibiotic resistance among tox+ and tox- E. coli strains isolated in Angeles City, Republic of the Philippines

<table>
<thead>
<tr>
<th>Population</th>
<th>Resistance (%)</th>
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<tbody>
<tr>
<td></td>
<td>Ap</td>
</tr>
<tr>
<td>Hostesses</td>
<td></td>
</tr>
<tr>
<td>tox+ E. coli (49)</td>
<td>40</td>
</tr>
<tr>
<td>tox- E. coli (1,500)</td>
<td>26</td>
</tr>
<tr>
<td>RWs</td>
<td></td>
</tr>
<tr>
<td>tox+ E. coli (25)</td>
<td>17</td>
</tr>
<tr>
<td>tox+ E. coli (680)</td>
<td>17</td>
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</tbody>
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* Ap, Ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Nm, neomycin; Sm, streptomycin; Su, sulfisoxazole; Tc, tetracycline.

The epidemicologic data on tox+ E. coli in the Philippines was not associated with an increase in the point prevalence of LT+ ST+ or LT- ST- E. coli.

No attempt was made to determine the prevalence of LT- ST+ E. coli since this would have required 49,740 suckling mice, which were not available. Recent studies (5, 14) suggest that culturing isolates in pools or pooling culture supernatants reduces the number of assays required. In experiments performed in this laboratory, however, both methods have occasionally failed to identify ST+ E. coli, especially when a small proportion of the isolates or their supernatants included in the pools are tox+ (B. Murray and P. Echeverria, in press). Therefore, individual colonies were tested for LT, and only LT+ isolates were tested for ST.

This study demonstrates that antibiotics did not increase the prevalence of LT+ ST+ or LT- ST- E. coli in the populations examined. Presumably, the prevalence of plasmids encoding for both antibiotic resistance and toxin production was low. Antibiotics other than penicillin and ampicillin (antibiotics which were used most frequently) might have exerted a stronger selective pressure on fecal enterobacteriaceae and perhaps selected for more widespread resistance and perhaps Ent. Interestingly, 1 of 12 hostesses infected with tetracycline-resistant LT+ ST- E. coli said that she had received tetracycline in the preceding week. In this study, antibiotic pressure which was extensive enough to result in the emergence of antibiotic resistance was not accompanied by an increase in Ent.

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LITERATURE CITED

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