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Epidemiology of Bacterial Pathogens Associated with Infectious Diarrhea in Djibouti

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During a survey examining the causes of diarrhea in the East African country of Djibouti, 140 bacterial pathogens were recovered from 209 diarrheal and 100 control stools. The following pathogens were isolated at comparable frequencies from both diarrheal and control stools: enteroadherent *Escherichia coli* (EAEC) (10.6 versus 13%), enterotoxigenic *E. coli* (ETEC) (11 versus 10%), enteropathogenic *E. coli* (EPEC) (7.7 versus 12%), *Salmonella* spp. (2.9 versus 3%), and *Campylobacter jejuni-C. coli* (3.3 versus 5%). Surprisingly, the EAEC strains isolated did not correspond to well-recognized EPEC serogroups. No *Yersinia* spp., enteroinvasive *E. coli*, or enterohemorrhagic *E. coli* were isolated during the course of this study. Only the following two genera were recovered from diarrheal stools exclusively: *Shigella* spp. (7.7%) and *Aeromonas hydrophila* group organisms (3.3%). *Shigella flexneri* was the most common *Shigella* species isolated. Patients with *Shigella* species were of a higher average age than were controls (27 versus 13 years), while subjects with *Campylobacter* or *Salmonella* species belonged to younger age groups (2.6 and 1.6 years, respectively). *Salmonella* cases were more often in females. *Shigella* diarrhea was associated with fecal blood or mucus and leukocytes. ETEC was not associated with nausea or vomiting. Anorexia, weight loss, and fever were associated with the isolation of *Salmonella* and *Aeromonas* species. EAEC, ETEC, EPEC, and *Shigella* species were resistant to most drugs used for treating diarrhea in Africa, while the antibiotic most active against all bacteria tested was norfloxacin. We conclude that in Djibouti in 1989, *Shigella* and *Aeromonas* species must be considered as potential pathogens whenever they are isolated from diarrheal stools and that norfloxacin should be considered the drug of choice in adults for treating severe shigellosis and for diarrhea prophylaxis in travelers.

The etiology and epidemiology of acute diarrheal disease in East Africa remain largely undefined. Numerous bacterial enteropathogens have been implicated in regional disease episodes (3, 11, 15, 17). However, their relative pathogenic potential appears to vary according to local geography, the epidemiological characteristics of the study site, and the resident populations involved. Thus, it is exceedingly difficult to generalize the public health significance of individual enteropathogens on a regional basis. Consequently, the Djiboutian Ministry of Health, with the support of the World Health Organization and the U.S. Naval Medical Research Unit No. 3, conducted a nationwide diarrhea survey in February 1989 to identify the types and prevalences of enteropathogenic bacteria associated with severe local diarrheal illness, as well as the epidemiological characteristics and susceptibility of these bacteria to antimicrobial agents.

MATERIALS AND METHODS

Study place and time, study population, and general study design. Djibouti is a small country in East Africa whose inhabitants belong to various ethnic communities. The Afars live in the north and southwest of the country, while the southeast is inhabited by Somalis. In addition to these two indigenous and predominant ethnic groups, many people presently living in Djibouti have ancestry in more remote regions, like Ethiopia, Yemen, India, and Europe.

The following study was conducted during the entire month of February 1989. During that time, the average daily minimum and maximum temperatures were 25 and 29.4°C, respectively. The average relative air humidity oscillated between 77 and 91%. Average daily atmospheric pressure was 1,013 millibars, and average insolation was 7.8 h/day. No rain fell during the study month.

The diarrhea survey involved individuals of both sexes, of all age groups, and of all ethnic communities living in Djibouti. Individuals were enrolled from seven health units in Djibouti City and from various rural locations throughout the country. Diarrhea was defined according to local cultural norms, i.e., individuals who presented to a health center with a complaint of diarrhea, regardless of associated symptoms. For every two subjects with diarrhea who met the definition of a study patient, one subject who did not complain of diarrhea, matched for age and habitat, was selected as a control.

All study and control subjects had an epidemiological and medical interview, in addition to a clinical examination, performed by a physician or other health worker fluent in the local languages. The epidemiological questionnaire and the medical workup contained 36 variables each. Results were entered into computerized data files.

Common statistical methods were used for analyzing the data, e.g., Student's *t* test for means and probability hypothesis tests for proportions.

Laboratory methods. Diarrhea patients and control subjects had stool specimens collected and processed in similar ways. Stool specimens or rectal swabs (in modified Cary-Blair medium) were cultured directly onto MacConkey agar, salmonella-shigella agar, Hektoen agar, thiosulfate-citrate-bile salts-sucrose agar, and into selenite F enrichment broth. Selenite broth was subcultured onto MacConkey, Hektoen, and salmonella-shigella agars after overnight incubation at 35°C. All plates were examined for *Salmonella* spp., *Shigella*

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TABLE 1. Comparison of general demographic and epidemiological characteristics among diarrhea patients and control subjects

Variable	Diarrheal patients	Controls	Statistics[a]
Sample size	209	100	
Male-to-female ratio	1.6	1.4	NS
Somali-to-Afar ratio	3.2	3.4	NS
Mean age in yr (SD)	13 (16)	13 (15)	NS
Mean no. people in household (SD)	6 (4)	7.6 (3.7)	NS
Mean no. rooms in house (SD)	2.1 (1.1)	2.2 (1.2)	NS
% With characteristic			
Education[b] of patient or father	38	42	NS
Consulting bush doctors	36	18	$P < 0.01$
Electricity at home	59	69	NS
Refrigerator at home	50	64	$P < 0.05$
Running tap water at home	69	74	NS
Indoor toilet at home	78	82	NS
Goats or sheep kept at home	12	10	NS
Cats kept at home	30	28	NS
Frequent consumption of:			
Milk	70	74	NS
Chicken	12	19	NS
Meat	47	57	NS
Fish	12	22	$P < 0.05$

[a] Means compared by Student's t test; difference between two proportions determined by hypothesis tests. NS, Not significant.
[b] Literate.

spp., and *Vibrio* spp. by standard microbiological methods, the API 20E system (Analytab Products, Plainview, N.Y.), and commercial antisera, if indicated (Difco Laboratories, Detroit, Mich.) (6). *Yersinia enterocolitica* isolation was attempted from the initial 100 stool samples by cold-temperature enrichment procedures. A portion of stool was inoculated into phosphate-buffered saline and incubated at 4°C for 3 weeks. Cold-temperature enrichment tubes were subcultured to cefsulodin-Irgasan-novobiocin agar (Difco) on a weekly basis (4). For the detection of *Aeromonas hydrophila* group organisms, stool samples were plated on sheep blood-ampicillin (30 μg/ml) agar (12) and incubated at 35°C for 18 to 24 h. Presumptive *Aeromonas* colonies were picked and tested for oxidase on filter paper saturated with 1% tetramethyl-*p*-phenylenediamine dihydrochloride. All oxidase-positive colonies were further identified by the API 20E system. For the detection of *Campylobacter jejuni-C. coli*, stools were cultured on Blaser's selective medium and inoculated at 42°C for 48 h under microaerophilic conditions (2). For investigating the diarrhea-producing potential of *Escherichia coli*, five presumptive colonies were picked from each specimen and were tested for the presence of genes encoding for the production of heat-stable (ST) or heat-labile (LT) enterotoxins or both with enzyme-labeled oligonucleotide DNA probes (13). Colonies were also tested for HEp-2 cell adherence in an attempt to detect localized, diffuse, or aggregative adherent strains (enteroadherent *E. coli* [EAEC]) (8, 16). Somatic serotyping of enteropathogenic *E. coli* (EPEC) was performed by the slide-agglutination method with Coli-Test Sera polyvalent I and polyvalent II (Behringwerke AG, Marburg, Federal Republic of Germany). If results were negative, *E. coli* colonies were submitted to Coli-Test Sera Anti-O25 and Anti-O44. In the event of positive reactions with polyvalent antisera, the colonies were tested with monovalent antisera against the following serogroups: O26, O55, O78, O86, O111, O114, O119, O124, O125, O126, O127, and O128. *E. coli* colonies were evaluated for enteroinvasiveness (enteroinvasive *E. coli*) by performing the Sereny test on nonmotile strains that were lysine decarboxylase negative (14). For the identification of enterohemorrhagic *E. coli* strains, all colonies were screened for growth on sorbitol-MacConkey agar supplemented with O157:H7 antiserum as previously described (7). Fecal smears, stained with methylene blue, were screened microscopically for fecal leukocytes.

All bacterial pathogens isolated during the course of this study were subsequently evaluated for antibiotic susceptibility by the Bauer-Kirby radial disk diffusion method with a battery of 14 different antimicrobial agents (1).

RESULTS

Origin of stool samples and general characteristics of study cases. A total of 309 stools were collected and cultured. Of these, 209 were from patients with diarrhea and 100 were from controls. Table 1 compares the general demographic and epidemiological characteristics of diarrhea patients and control subjects. Control and diarrhea cases were fairly well matched. However, controls may have belonged to slightly higher social groups than their ill counterparts, since a significantly higher proportion of controls had a refrigerator at home, ate fish regularly, and claimed not to consult traditional healers (Table 1).

Enteric bacteria isolated. A total of 140 potential bacterial pathogens were isolated from the 309 stools examined; a total of 108 (35%) stools yielded a single enteric bacterial pathogen, while 16 (5%) stools yielded two potential etiologic agents. EAEC was the pathogen most commonly recovered (35 isolates); 19 exhibited aggregative type adherence on HEp-2 cells, 10 exhibited localized adherence, and 6

TABLE 2. Distribution of bacterial pathogens isolated from diarrheic and control stools during February 1989 in Djibouti

Bacterial pathogen (characteristics)	No. of diarrheal stools (n = 209)	No. of control stools (n = 100)
EAEC	22 (10.6)[a]	13
AA	13	6
LA	4	6
DA	5	1
ETEC	23 (11.0)	10
LT	10	6
ST	10[b]	3
LT and ST	3	1
EPEC	16 (7.7)	12
Shigella spp.[c]	16 (7.7)	0
C. jejuni-C. coli	7 (3.3)	5
Salmonella spp.[d]	6 (2.9)	3
A. hydrophila	7 (3.3)	0

[a] Number in parentheses gives the percent positivity for diarrheal stools.
[b] Serogroup O78, two; serogroup O114, one; serogroup O128, one.
[c] *S. dysenteriae*, 2; *S. flexneri*, 12; *S. boydii*, 2.
[d] Serogroup B, one; serogroup C_1, four; serogroup D_1 (nontyphoidal), two; *S. typhi*, one; serogroup E, one.

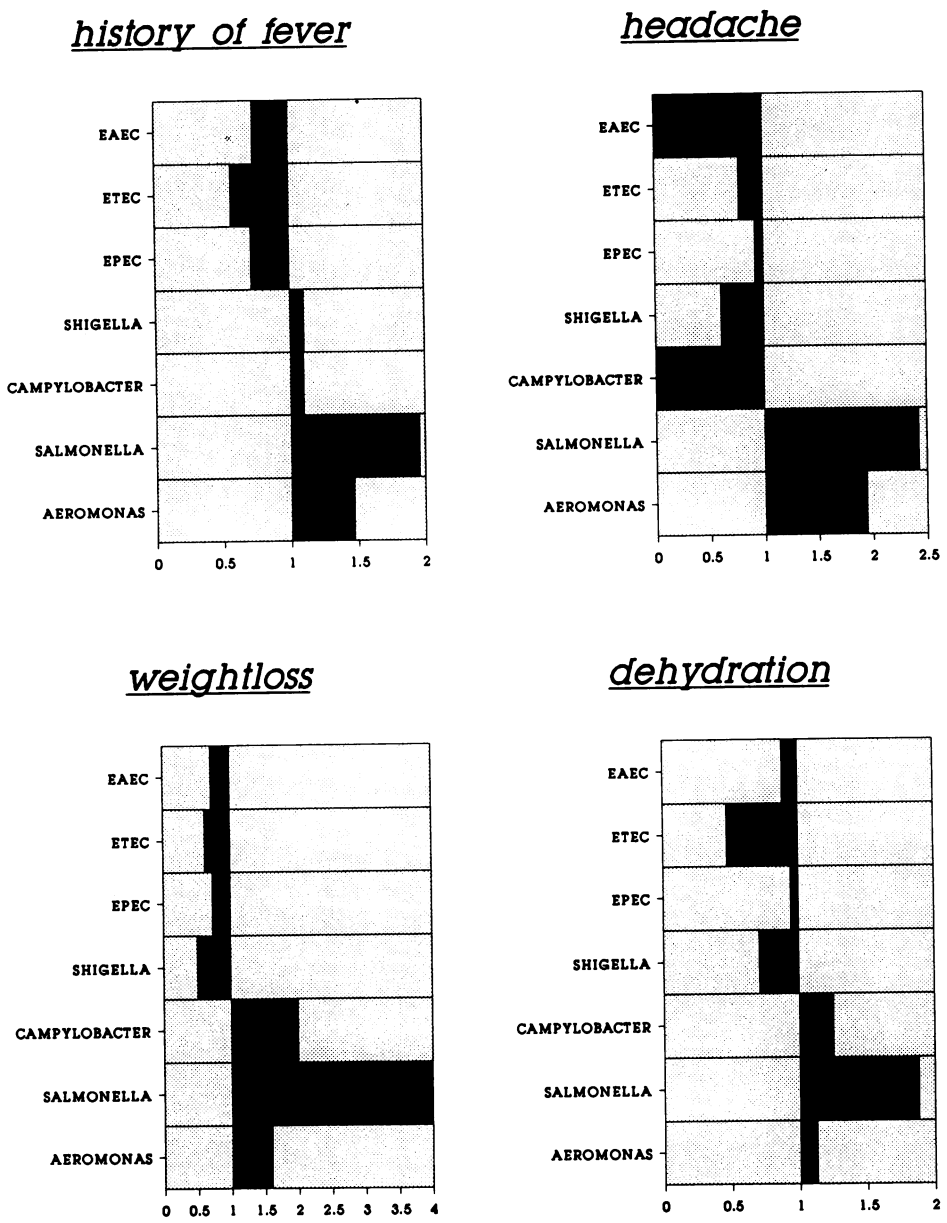

FIG. 1. Associations between eight selected symptoms and signs and the seven diarrheal pathogens isolated in Djibouti. The ratios depicted correspond to the frequency of particular symptoms and signs among infected individuals divided by the frequency of the same symptoms and signs in the entire group of diarrheal patients. Absent associations are indicated by short columns (ratio risk close to 1). The higher the ratio, the farther the column to the right, the greater the positive association; the lower the ratio, the farther the column to the left, the greater the negative association.

exhibited diffuse adherence. The EAEC strains isolated from both diarrheal and control stools did not belong to any of the EPEC serogroups frequently associated with this phenotype. Enterotoxigenic *E. coli* (ETEC) was recovered from 33 stools; LT-producing ETEC was recovered from 16 stools, ST-producing ETEC was recovered from 13 stools, and strains producing both ST and LT were recovered from 4 stools. EPEC was recovered from 28 stools. EPEC serogroups O25, O26, O78, and O86 were detected in both patients and controls, while serogroups O44, O124, and O127 were detected in controls only, and serogroups O119, O114, and O128 were detected in patients only. Seven percent of the EPEC strains isolated from patients and controls exhibited adherence to HEp-2 cells. Two strains of serogroup O78, one strain of serogroup O114, and one strain of serogroup O128, all isolated from diarrheal patients, were also ST positive. *Shigella* spp. were isolated from 16, *C. jejuni-C. coli* from 12, *Salmonella* spp. from 9, and *A. hydrophila* from 7 stools, respectively.

The distribution of bacterial pathogens recovered from patients and controls indicates that most enteric pathogens were isolated at similar frequencies from both diarrheal and control stools (Table 2). This finding was also apparent when the double infections were analyzed alone, since 12 (5.7%) double infections occurred among patients and 4 occurred among controls. Only two enteropathogens, *Shigella* spp. and *A. hydrophila*, were isolated exclusively from diarrheal stools. No *Y. enterocolitica*, *Vibrio cholerae*, enteroinvasive

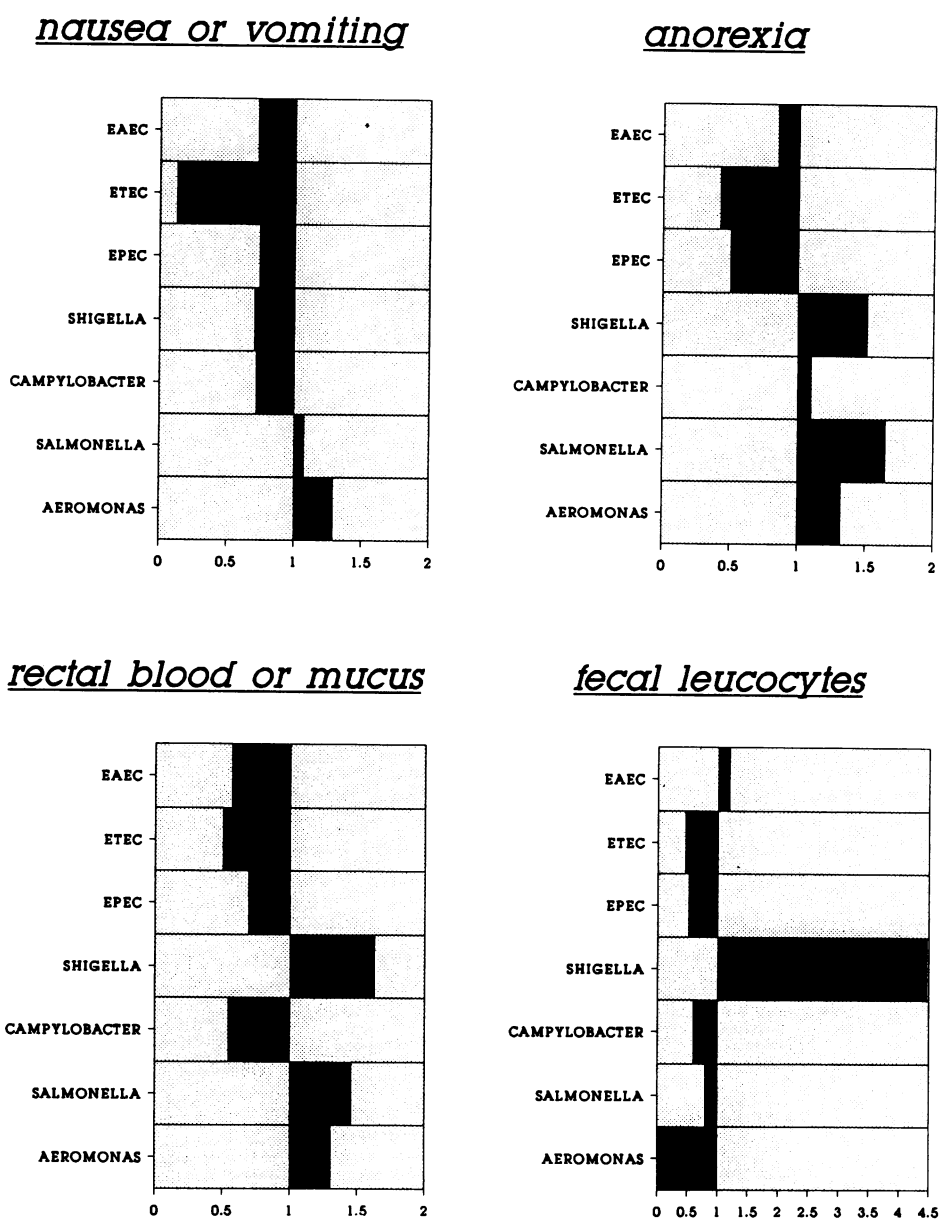

FIG. 1—*Continued.*

E. coli, or enterohemorrhagic *E. coli* were isolated from either patients or controls during the survey.

Epidemiological characteristics associated with individual enteropathogens. When potential risk factors were analyzed by particular pathogens, a number of interesting associations were recognized. In particular, *Shigella* patients belonged to the Afar ethnic group more often than expected (odds ratio, 2.7; $P < 0.01$) and they tended to keep animals in their homes more often than did controls (25% kept sheep or goats at home while only 10% of controls did; $P = 0.05$). *Shigella* patients were also of a higher average age (27 years) than were controls, while subjects with positive stool cultures for *Campylobacter* (3 years) or *Salmonella* (2 years) species belonged to younger age groups than did controls (13 years). Interestingly, *Salmonella* cases were more often female (odds ratio, 1.9; $P < 0.05$) and tended more often to be members of uneducated families (odds ratio, 1.7; $P = 0.05$). Subjects with stool cultures positive for ETEC or *Campylobacter* species tended to have electricity less frequently at home (42 and 18% respectively) when compared with controls (69%; both, $P < 0.01$) and subjects positive for all other enteropathogens. Patients with *Aeromonas* infection belonged to significantly smaller households than did controls (4.8 versus 7.6 persons per family; $P < 0.01$), despite a similar number of rooms in their homes. Lastly, individuals infected with enteropathogens were found to be comparable to individuals that tested negative in their access to indoor toilet facilities and tap water.

Semeiology according to individual enteropathogens. Diarrheal patients, as a group, tended to seek medical care for their illness at approximately 10 days postonset. In contrast, patients with diarrhea caused by *Shigella* or *Aeromonas* species tended to seek care after only 5 and 7 days, respectively (differences not significant), possibly because these

TABLE 3. Antibiotic susceptibility patterns of bacterial enteropathogens isolated in Djibouti during February 1989[a]

Isolates and characteristic (no. tested)	% Resistant to antibiotics[b]													
	AN	AM	ATM	CRO	CR	C	DO	E	GM	NA	N	G	TE	SXT
EAEC (35)[c]														
AA (19)	0	68	5	0	68	21	79	100	0	5	0	53	74	37
LA (10)	0	80	10	0	70	20	80	90	0	0	0	50	60	50
DA (06)	0	67	0	0	17	50	83	83	0	0	0	67	83	67
ETEC (33)														
LT^+ (16)	0	44	0	0	42	19	75	100	0	0	0	50	63	38
ST^+ (13)	0	77	0	0	85	8	85	100	0	0	0	77	85	62
LT^+-ST^+ (4)	0	25	0	0	75	0	25	100	0	0	0	25	25	25
EPEC (28)	0	46	4	0	46	29	71	100	0	4	0	50	71	36
Shigella spp. (16)	0	69	6	0	47	75	94	50	0	0	0	100	94	69
A. hydrophila (7)	0	100	0	0	100	0	0	71	0	0	0	100	14	29
C. jejuni-C. coli (12)	0	67	100	92	100	0	0	0	0	0	0	90	8	100
Salmonella spp. (9)	0	22	0	0	0	0	0	100	0	0	0	56	0	11

[a] Isolates from both diarrhea patients and controls are included.
[b] Abbreviations: AN, amikacin; AM, ampicillin; ATM, aztreonam; CRO, ceftriaxone; CR, cephalosporin; C, chloramphenicol; DO, doxycycline; E, erythromycin; GM, gentamicin; NA, nalidixic acid; N, norfloxacin; G, sulfisoxazole; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole.
[c] Abbreviations: AA, aggregative adherence; LA, localized adherence; DA, diffuse adherence.

diarrheas were fairly severe, prompting patients to seek medical help earlier after onset. On the average, diarrhea patients passed 6 stools per day (standard deviation, 3) and no notable difference was apparent when further analysis by pathogen isolated was attempted. Figure 1 summarizes the associations of selected signs and symptoms in individuals infected with particular enteric pathogens. Of particular interest was the positive association of *Shigella* diarrhea with a history of fecal blood or mucus and the microscopic presence of fecal leukocytes; the isolation of ETEC, but not EAEC and EPEC, was negatively associated with nausea or vomiting. A negative association also existed between anorexia and the isolation of both ETEC and EPEC, but not EAEC. Finally, anorexia, weight loss, and fever were positively associated with the isolation of both *Salmonella* and *Aeromonas* species.

In vitro antibiotic susceptibility. Table 3 illustrates the antibiotic susceptibility patterns of the 140 bacterial enteropathogens recovered from both patients and controls during the survey. Essentially, all isolates were susceptible to norfloxacin, amikacin, and gentamicin. With the exception of *Campylobacter* strains, all isolates were also 100% susceptible to ceftriaxone (broad-spectrum cephalosporin), while more than 90% were also susceptible to aztreonam. EAEC, ETEC, EPEC, and *Shigella* species were highly resistant to most antimicrobial agents commonly used for treating diarrhea in Africa (e.g., ampicillin, tetracyclines, and trimethoprim-sulfamethoxazole), while only 22 and 11% of the *Salmonella* isolates were resistant to ampicillin and trimethoprim-sulfamethoxazole, respectively. Finally, erythromycin was active only against *Campylobacter* isolates.

DISCUSSION

The goal of the present study was to identify bacterial pathogens associated with infectious diarrhea in Djibouti. The most interesting finding was that the majority of enteric isolates occurred at very comparable frequencies in both diarrheal and control stools. These isolates comprised ETEC, EPEC, EAEC, *Salmonella* spp., and *C. jejuni-C. coli*. Only two bacterial enteropathogens, *Shigella* spp. and *A. hydrophila*, were recovered exclusively from diarrheal stools. Therefore, these two enteric agents should be considered pathogenic whenever isolated from diarrhea stools in Djibouti. Conversely, more caution seems indicated for interpreting the pathogenic role of the remaining enteric bacteria encountered in this survey. Indeed, when these bacteria are isolated from diarrheal stools, questions arise as to whether they are the actual cause of the illness, whether they are purely commensals, or whether the infected individuals are healthy carriers. Nonetheless, these bacteria must be considered as potential causes of diarrhea in nonimmune travelers to Djibouti.

Our findings are in direct contrast with reports published from the following two countries that have common borders with Djibouti: Ethiopia to the northwest and Somalia to the south. In these countries, most enteropathogens studied were more frequently isolated from diarrheal patients than from asymptomatic controls (3, 15). However, comparisons between our survey and those reports are difficult, since their study populations included only pediatric age groups that were followed over several years, while all age groups were included in our present point prevalence study.

The ETEC isolation rate in Djibouti was astonishingly similar to the rate described in Somalia (11%), but we did not observe a decrease in the ETEC isolation rate with increasing age, as was described in Mogadishu (3). Our findings concerning *Shigella* diarrhea in Djibouti were also similar to the *Shigella* reports from Somalia, in matters of species prevalence (predominance of *S. flexneri*) and positive association with fecal leukocytes on stool microscopy. *Campylobacter* isolation rates in Djibouti were about half the rates observed in Somalia (8%). However, the same trend existed in Djibouti and Somalia in that this organism was isolated with equal frequency in both symptomatic and asymptomatic individuals. As suggested in the Somalia study, we also consider *Aeromonas* species to be enteric pathogens whenever isolated from diarrheal stools in this region.

Our Djibouti report presents the first description of EAEC in East Africa. It is noteworthy that EAEC isolated from both diarrheal and control stools did not belong to recog-

nized EPEC serogroups. Conversely, the EPEC isolates from patients and controls were nonadherent, with the exception of two strains belonging to serogroup O25, one diarrheal isolate exhibiting localized adherence and one control isolate exhibiting diffuse adherence. Hence, it appears that in Djibouti, although EAEC and EPEC strains were isolated at a similar frequency from both diarrheal and control stools, they may, in fact, belong to quite distinct groups. Moreover, our data on EAEC and EPEC seem to support suggestions from Mexico that HEp-2 adherence is a virulence characteristic that is independent of EPEC serotype (9, 10). This conclusion is not supported by a recent report from Brazil describing 80% of colonies exhibiting localized adherence as belonging to traditional EPEC serotypes (5). However, these differences could still be explained by regional differences in the prevalence of these two phenotypic markers.

With the exception of *Salmonella* spp., the enteric bacteria prevalent in Djibouti displayed high levels of in vitro resistance to the antimicrobial drugs commonly used to treat infectious diarrheas in Africa. Our data suggest that, if treatment is warranted for *Shigella* disease or if diarrhea prophylaxis is indicated for travelers, the new quinolone, norfloxacin, should be considered the drug of choice for adults in Djibouti. Indeed, norfloxacin was found active against all 140 bacterial isolates tested in our series, and this drug has recently been shown to be a safe and effective prophylactic agent against traveler's diarrhea in Egypt (D. A. Scott, R. L. Haberberger, S. A. Thornton, and K. C. Hyams, Am. J. Trop. Med. Hyg., in press). However, we are still confronted with a dilemma as to what drug to use for diarrheic children in need of supplemental antibiotic therapy in this area of East Africa.

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