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# Antibiotic Resistance in Enterotoxigenic and Non-Enterotoxigenic *Escherichia coli*

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Antibiotic disk susceptibility tests were done on 220 strains of *Escherichia coli* belonging to serotypes reported in the literature to be associated with the production of enterotoxin. A total of 128 (58%) were resistant to one or more antibiotics, sulfa drugs, or chemotherapeutic agents. An analysis of these strains revealed primary, secondary, and tertiary drug resistance patterns that indicated a selective pattern in the formation of multiple drug resistance in *E. coli*. Resistances to certain antibiotics were more likely to occur in pairs and triads (secondary resistance patterns) that were often combined or coexisted in a single strain of *E. coli* to produce tertiary drug resistance patterns, conferring drug resistance to five or six different antibiotics. Among enterotoxin-associated serotypes, single and multiple drug resistance was less frequently associated with enterotoxin-producing strains than with strains from the same serotype that were not enterotoxigenic. Within the enterotoxigenic *E. coli*, single and multiple resistance to antibiotics was more frequent in strains producing only heat-stable enterotoxin (ST) than in strains producing only heat-labile enterotoxin (LT) or both. The number of resistances to different antibiotics per resistant strain averaged approximately 1.4 for LT plus ST or LT strains, and 3.9 for ST strains and nonenterotoxigenic strains. Phenotypic characterization of 170 strains for four usually plasmid-mediated characteristics showed that the number of antibiotics to which a strain was directly resistant varied with the type and number of plasmid-mediated characteristics present.

Enterotoxigenic *Escherichia coli* (ETEC) have been implicated in sporadic cases and epidemic outbreaks of diarrhea in both infants and adults in many parts of the world (4, 21, 29, 31, 33). ETEC produce one or both of two plasmid-mediated enterotoxins (18, 37): a heat-stable enterotoxin (ST) and a heat-labile enterotoxin (LT). The plasmid genes that control enterotoxin production occupy only a small portion of the total plasmid genome (7, 39). To delineate pathogenic mechanisms and their transmissibility in ETEC, it would be useful to study other traditionally plasmid-mediated characteristics associated or compatible with the enterotoxin (Ent) plasmids. Other plasmids that might coexist in the same cell with Ent include those with genes coding for antibiotic resistance (1, 3, 17, 19, 20, 41), colonization factors (CFs) (13, 14, 26, 38), and a filterable, heat-labile hemolytic entity (HLY1) (16, 25, 37). Antibiotic disk susceptibility tests were done on 220 strains of *E. coli* belonging to serotypes previously reported in the literature to be associated with enterotoxin production (enterotoxin-associated serotypes). This study was undertaken to determine the prevalence of antibiotic resistance in these strains and to investigate possible associations between antibiotic resistance and other plasmid-mediated characteristics indicating virulence.

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## MATERIALS AND METHODS

All 220 strains of *E. coli* were received by the Enteric Section, Bacteriology Division, Center for Disease Control between 1960 and 1978. They were initially rejuvenated by culturing on Trypticase soy agar plates before being transferred to and maintained on blood agar base slants at room temperature.

Antibiotic susceptibility tests for 12 antibiotics were done according to the standard disk diffusion techniques recommended in the *Manual of Clinical Microbiology* (22). The disks (BBL Sensi-disk) used were: colistin (CL10), streptomycin (S10), cephalothin (CF30), gentamicin (GM10), nalidixic acid (NA30), kanamycin (K30), tetracycline (TE30), chloramphenicol (C30), rifampin (RA5), ampicillin (AM10), carbenicillin (CB100), and sulfadiazine (SD250). These anti-

biotics were chosen on the basis of their clinical and genetic relevance and their use as epidemiological markers.

Of the 220 enterotoxin-associated serotype strains, 170 were assayed for four plasmid-mediated characteristics: ST, LT, CFs, and a filterable, heat-labile hemolytic activity (HLY1). The tests for ST and LT were the infant mouse assay (8, 15) and the Y1 adrenal cell assay (10, 30), respectively. The assays for CFs consisted of hemagglutination tests with human and bovine erythrocytes (11, 12, 14, 27) and a micro-precipitation test (C. F. Deneke, G. M. Thorne, and S. L. Gorbach, Abstr. 15th Joint Conf. on Cholera, U. S.-Japan Coop. Med. Sci. Prog. 1979, p. 35–36) with specific anti-pili sera (9) that were supplied by Grace Thorne, New England Medical Center, Boston, Mass. Screening tests for hemolytic activity were done in liquid saline-peptone medium and in horse erythrocyte agar plates according to the methods of Cooke (6), and a specific assay for the presence of HLY1 was done, with minor modifications (8a), according to the basic procedures of Smith (36) and Short and Kurtz (34).

## RESULTS

Of 220 strains of *E. coli* belonging to enterotoxin-associated serotypes, 128 (58%) were resistant to at least 1 of 12 antibiotics. The number of strains resistant to a single antibiotic ranged from 65 (30%) for sulfadiazine to none for gentamicin and colistin. A survey of multiple drug resistance among these 128 strains provided the data in Fig. 1 and 2. In Fig. 1 the conditional probability of finding resistance to a second antibiotic (listed vertically), when resistance to a first antibiotic (listed horizontally) already exists, is given for any pair of antibiotics both as a fraction (number of strains resistant to both the first and second antibiotics divided by the number of strains resistant to only the first antibiotic) and as a percentage. A panorama of the multiple drug resistance associations found among all possible pairs of antibiotics in multiply drug-resistant strains is presented in Fig. 2. The major antibiotic resistance patterns possessed by multiply resistant strains are presented in Fig. 3. Excluding the usually chromosomally mediated drug resistance to rifampin and cephalothin left 85 strains that were resistant to 2 or more of 10 additional antibiotics. Of these 85, 65 (76%) were multiply resistant to two or more antibiotics.

When all 220 test strains were sorted into groups based on enterotoxin production, and the percentages of strains resistant to individual antibiotics were compared (Table 1), the group of non-ETEC had higher percentages of drug-resistant strains than did any group containing ETEC. A subset of 65 human strains isolated in the United States were similarly sorted and compared (Table 2). Again the group of non-ETEC possessed higher percentages of drug-resistant strains than did any group containing ETEC. The ETEC in this subset were found to harbor significantly fewer strains expressing resistance to three of eight antibiotics (Table 2). No ETEC resistant to colistin, nalidixic acid, cephalothin, or gentamicin were available for comparison in this table.

Among the ETEC, strains producing ST alone expressed drug resistances to more antibiotics than did strains producing LT (Table 1). Further comparison of antibiotic resistance with enterotoxigenicity (Table 3) showed that the average number of antibiotic resistances per resistant strain was much lower for strains producing LT plus ST or LT alone than for strains producing ST alone or neither enterotoxin. ETEC producing ST alone were resistant to an average of four different antibiotics. This average was as high as that seen in the non-ETEC.

Table 4 compares antibiotic resistances among 170 strains of *E. coli* phenotypically characterized by the presence or absence of ST, LT, CFs, and HLY1. A high ratio of antibiotic resistances to resistant strains was found in strains lacking all four plasmid-mediated characteristics and in strains with the following phenotypic patterns: ST, CF, and CF HLY1. Little drug resistance was found in strains with patterns possessing either LT (e.g., ST LT CF, ST LT, LT CF, or LT alone) or CF in conjunction with an Ent plasmid (e.g., ST LT CF, ST CF, or LT CF).

## DISCUSSION

Seventy-six percent (65/85) of our resistant *E. coli* were resistant to 2 or more of 10 antibiotics. This data agrees with similar data recently reported from Canada, where 60 to 70% of resistant *E. coli* were found to be insensitive to two or more separate antibiotics (G. Bezanson and H. Lior, Laboratory Center for Disease Control Newsletter 1979-2, p. 15).

An analysis of all 128 strains of *E. coli* showing single or multiple drug resistance yielded the primary, secondary, and tertiary drug resistance patterns listed in Fig. 3, excluding an obviously longer list of tertiary patterns. Our data indicate that selection occurs in the formation of multiple drug resistance in *E. coli*. Figures 1 and 2 show that secondary drug resistance patterns were most likely to occur between certain pairs of antibiotics. For example, when resistance to sulfadiazine was present, 69% of all sulfadiazine-resistant strains were also resistant to tetracycline, and 71% of all tetracycline-resistant strains were also sulfadiazine resistant (e.g., in Fig. 1: TE given SD = 69%, SD given TE = 71%, CM given KM = 67%, KM given CM = 75%). Other

| | CF | AM | CB | CM | KM | SD | SM | TE | RA |
|---|---|---|---|---|---|---|---|---|---|
| RA[a] given | 0/4 | 15/40 | 15/35 | 4/24 | 3/18 | 16/65 | 15/49 | 16/63 | 64/64 |
| %[b] | 0 | 38 | 43 | 17 | 17 | 25 | 31 | 25 | 100 |
| TE given | 1/4 | 31/40 | 30/35 | 22/24 | 15/18 | 45/65 | 41/49 | 63/63 | 16/64 |
| % | 25 | 78 | 86 | 92 | 83 | 69 | 84 | 100 | 25 |
| SM given | 1/4 | 28/40 | 28/35 | 18/24 | 12/18 | 42/65 | 49/49 | 42/63 | 14/64 |
| % | 25 | 70 | 80 | 75 | 67 | 65 | 100 | 67 | 22 |
| SD given | 1/4 | 34/40 | 32/35 | 22/24 | 16/18 | 65/65 | 42/49 | 45/63 | 16/64 |
| % | 25 | 85 | 91 | 92 | 89 | 100 | 86 | 71 | 25 |
| KM given | 0/4 | 8/40 | 8/35 | 18/24 | 18/18 | 16/65 | 11/49 | 15/63 | 3/64 |
| % | 0 | 20 | 23 | 75 | 100 | 25 | 22 | 24 | 5 |
| CM given | 0/4 | 13/40 | 13/35 | 24/24 | 12/18 | 22/65 | 18/49 | 22/63 | 4/64 |
| % | 0 | 32 | 37 | 100 | 67 | 34 | 37 | 35 | 6 |
| CB given | 1/4 | 35/40 | 35/35 | 13/24 | 8/18 | 32/65 | 28/49 | 30/63 | 14/64 |
| % | 25 | 88 | 100 | 54 | 44 | 49 | 57 | 48 | 22 |
| AM given | 4/4 | 40/40 | 35/35 | 13/24 | 8/18 | 34/65 | 28/49 | 30/63 | 15/64 |
| % | 100 | 100 | 100 | 54 | 44 | 52 | 57 | 48 | 23 |
| CF[c] given | 4/4 | 4/40 | 1/35 | 0/24 | 0/18 | 1/65 | 1/49 | 1/63 | 0/64 |
| % | 100 | 10 | 3 | 0 | 0 | 2 | 2 | 2 | 0 |

[a]RA (rifampin), TE (tetracycline), SM (streptomycin), SD (sulfadiazine), KM (kanamycin), CM (chloramphenicol), CB (carbenicillin), AM (ampicillin), CF (cephalothin).

[b]Examples of %: CB given AM = 88% (out of 40 ampicillin-resistant strains, 35 or 88% were also carbenicillin resistant); AM given CB = 100%; SD given KM = 89%; KM given SD = 25%.

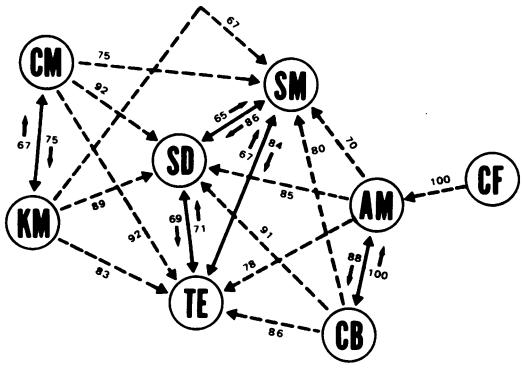
[c]Percentages of strains resistant to CF and RA (outer borders) serve as baseline percentages since resistance to these antibiotics is usually chromosomally mediated.

FIG. 1. *Antibiotic resistance associations between paired antibiotics in multiply resistant E. coli.*

pairs, however, were found to be present most often only when a particular one of the two antibiotic resistances was present. For example, when resistance to chloramphenicol was present, 92% of all chloramphenicol-resistant strains were also resistant to sulfadiazine, but only 34% of all sulfadiazine-resistant strains were also chloramphenicol resistant (e.g., in Fig. 1: SD given CM = 92%, but CM given SD = 34%; TE given KM = 83%, but KM given TE = 24%). Certain types of plasmids may contain recombination or insertion sites for particular antibiotic resistance genes. Furthermore, certain strains or serotypes of *E. coli* may readily accept and maintain specific plasmids, or plasmids in general. This could account for the apparent selection seen in the formation of multiple drug resistance in particular strains of *E. coli*.

Secondary resistance patterns often combined or coexisted in a single *E. coli* to yield the tertiary resistance patterns listed in Fig. 3. Whether the accumulation of plasmid-mediated drug resistances to form large resistance patterns is due to the accession of separate but compatible plasmids each containing individual drug resistance(s), or to an increase in the number of transposable elements, each carrying a gene(s) for drug resistance, within a single plasmid, is not fully known. Nonetheless, it would seem logical that the larger the complement of plasmid DNA possessed by a single cell, the greater chance there is for insertion of drug resistance transposons at sites within the plasmid(s).

Sack has stated (32) that ETEC, particularly

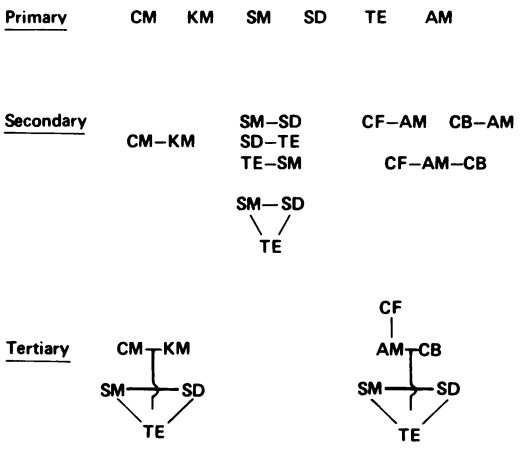



X--75-->Y (Resistance to Y was present in 75% of strains resistant to X)

X<91/67>Y (Resistance to Y was present in 91% of strains resistant to X; and resistance to X was present in 67% of strains resistant to Y)

AM = Ampicillin
CB = Carbenicillin
CF = Cephalothin
CM = Chloramphenicol
KM = Kanamycin
SD = Sulfadiazine
SM = Streptomycin
TE = Tetracycline

FIG. 2. *Panorama of antibiotic resistance associations between paired antibiotics in multiply resistant E. coli.*




AM = Ampicillin
CB = Carbenicillin
CF = Cephalothin
CM = Chloramphenicol
KM = Kanamycin
SD = Sulfadiazine
SM = Streptomycin
TE = Tetracycline

FIG. 3. *Primary, secondary, and tertiary antibiotic resistance patterns found among 128 strains of singly and multiply resistant E. coli. Connecting lines join multiple resistances.*

those strains that produce either ST plus LT or LT alone, are unusually susceptible to antibiotics. The data presented in Tables 1 to 4 support his statement. For three of the eight antibiotics listed in Table 2 there were significantly fewer drug resistances associated with ETEC than with non-ETEC. And, as Tables 3 and 4 show, among ETEC there was much less multiple drug resistance associated with strains producing LT plus ST, or LT alone, than with strains producing ST alone.

All of these data suggest possible interactions and incompatibilities among resistance genes, Ent genes, and the entire plasmid complement. Genotypic studies are presently under way to delineate some of these possible interactions; however, some observations can be reported at this time. Eleven strains lacking all four plasmid-mediated characteristics still possessed a high ratio of drug resistances per resistant strain (Table 4). These drug resistances are probably carried on otherwise cryptic plasmids which will be evident with transfer studies and a plasmid profile. Some antibiotic resistance genes are able to move from genome to genome by a nonclassical recombination event called transposition (5). It is not surprising that we found more drug resistances among ST-producing strains, since ST has recently been reported (40) on a transposon flanked by IS1 elements, an insertion sequence homologous to the inverted repeats flanking Tn*9*, the transposon known to carry chloramphenicol resistance (24). We surmise that a plasmid capable of accepting (inserting) ST into its genome would also be capable of inserting the gene(s) for chloramphenicol or possibly for other drug resistances. This could form a single plasmid coding for both enterotoxin production and drug resistance. McConnell et al. (23) recently reported such a case in a human isolate of *E. coli* which possessed a plasmid coding for ST, LT, and resistance to ampicillin. In addition, strains possessing an ST-mediating plasmid might be more likely to accept or maintain not only drug resistance plasmids but also other compatible plasmids, such as CF and HLY1 (Table 4).

In ETEC-producing LT, however, we found few strains resistant to antibiotics (Tables 2 to 4). The exception was sulfadiazine (Table 1), which appears to be on a separate plasmid compatible with the LT Ent plasmid (V. Baselski, personal communication). The low number of antibiotic resistances associated with LT strains of *E. coli* could be due to some basic incompatibilities between LT Ent and R plasmids, or there may be other mechanisms within the LT Ent plasmid itself that are responsible. For ex-

TABLE 1. *Antibiotic resistance data[a] for 220 E. coli strains belonging to enterotoxin-associated serotypes[b]*

| Antibiotic | No. of strains resistant (%) | | | | |
|---|---|---|---|---|---|
| | All strains ($n$ = 220)[c] | ST LT ($n$ = 17) | LT ($n$ = 15) | ST ($n$ = 27) | Non-ETEC ($n$ = 161) |
| Sulfadiazine | 65 (30) | 1 (6) | 7 (47) | 4 (15) | 53 (33) |
| Tetracycline | 64 (29) | 2 (12) | 2 (13) | 6 (22) | 54 (34) |
| Rifampin | 62 (28) | 4 (24) | 3 (20) | 5 (18) | 50 (31) |
| Streptomycin | 49 (22) | 0 (0) | 1 (7) | 3 (11) | 45 (28) |
| Ampicillin | 40 (18) | 0 (0) | 0 (0) | 2 (7) | 38 (24) |
| Carbenicillin | 35 (16) | 0 (0) | 0 (0) | 2 (7) | 33 (20) |
| Chloramphenicol | 24 (11) | 1 (6) | 0 (0) | 6 (22) | 17 (11) |
| Kanamycin | 18 (8) | 0 (0) | 0 (0) | 5 (18) | 13 (8) |
| Cephalothin | 4 (2) | 0 (0) | 0 (0) | 0 (0) | 4 (2) |
| Nalidixic acid | 2 (1) | 0 (0) | 0 (0) | 0 (0) | 2 (1) |
| Gentamicin | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Colistin | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

[a] Antibiotic susceptibility tests used BBL Sensi-disks CL10, S10, CF30, GM10, NA30, K30, TE30, C30, RA5, AM10, CB100, and SD250.

[b] The enterotoxin-associated serotypes tested consisted of the following and were reported in the literature, on at least two occasions before July 1978, to contain enterotoxigenic strains: O6:H16, O6:H-, O8:H9, O8:H?, O15:H11, O15:H-, O20:H-, O25:H-, O27:H20, O78:H11, O78:H12, O128:H7, O128:H12, O128:H21, O148:H28, and O149:H19.

[c] $n$, Number of strains tested.

TABLE 2. *Antibiotic resistances in 65 human E. coli strains isolated within the United States between 1960 and 1978*

| Antibiotic resistance[a] | No. of strains resistant (%) | | Probability of chance[b] |
|---|---|---|---|
| | ETEC ($n$ = 28) | Non-ETEC ($n$ = 37) | |
| Tetracycline | 6 (21) | 17 (46) | NS |
| Streptomycin | 3 (11) | 16 (43) | $\chi^2 = 6.66$, $P < 0.01$ |
| Rifampin | 7 (25) | 15 (40) | NS |
| Sulfadiazine | 7 (25) | 14 (38) | NS |
| Ampicillin | 1 (4) | 13 (35) | $\chi^2 = 7.62$, $P < 0.01$ |
| Carbenicillin | 1 (4) | 13 (35) | $\chi^2 = 7.62$, $P < 0.01$ |
| Chloramphenicol | 4 (14) | 7 (19) | NS |
| Kanamycin | 2 (7) | 5 (14) | NS |

[a] No ETEC were found to be resistant to colistin, nalidixic acid, cephalothin, or gentamicin; no non-ETEC were found to be resistant to colistin or gentamicin.

[b] Probability that the observed difference is due to chance alone. All probabilities are based on two-sample chi-square tests for significance. NS, Not significant.

TABLE 3. *Antibiotic resistance of 85 drug-resistant E. coli strains, by enterotoxin production*

| Enterotoxin produced | Total no. of antibiotics to which strains are resistant[b] | Total no. of resistant strains | Avg no. of resistances per strain |
|---|---|---|---|
| ST LT | 4 | 3 | 1.33 |
| LT | 10 | 7 | 1.42 |
| ST | 28 | 7 | 4.00 |
| Non-ETEC | 255 | 68 | 3.75 |

[a] Totals do not include resistance to rifampin or cephalothin because resistance to these drugs is usually chromosomally rather than plasmid mediated.

ample, an LT Ent plasmid may lack the necessary insertion site(s) for drug resistance transposons, or if LT itself were a transposon, it could possibly prevent acquisition of a second transposon (28), a mechanism referred to as transposition immunity (2). It is also possible that LT Ent plasmids do insert transposons for drug resistance and that such insertions interfere with the expression of the LT gene. Regardless of mechanism, it would appear that there is less risk of accumulating drug-resistant ETEC when LT-induced, rather than ST-induced, diarrhea is treated with antibiotics. However, it is presently impractical for most clinical laboratories to determine which toxin or toxin genes are responsible for a case of diarrhea. Therefore, there is no way to adequately differentiate between LT- and ST-induced cases for treatment purposes.

This discussion has important implications for the treatment of ETEC diarrhea with antibiotics. Since all ETEC possess at least one plasmid (Ent), which has been demonstrated on at least one occasion (23) to carry drug resistance genes, it is possible that dissemination of Ent could occur under the force of selective antibiotic pressure, either in the laboratory or in therapy (29). CF plasmids are often found associated with ETEC, and, from the data presented in Table 4, it appears that this additional plasmid may also harbor genes for drug resistance.

TABLE 4. *Antibiotic resistance[a] among 170 phenotypically characterized E. coli strains sorted by phenotypic pattern*

| Pattern | No. of resistant strains/no. of strains tested (%) | Total no. of antibiotic resistances/ no. of strains | Avg no. of resistances per strain |
|---|---|---|---|
| ST LT CF | 0/6 (0) | 0/0 | 0.00 |
| ST LT | 3/12 (25) | 4/3 | 1.33 |
| LT CF | 5/8 (62) | 7/5 | 1.40 |
| LT | 2/7 (29) | 3/2 | 1.50 |
| ST CF | 1/8 (12) | 2/1 | 2.00 |
| HLY1 | 6/14 (43) | 12/6 | 2.00 |
| None[b] | 11/37 (30) | 38/11 | 3.45 |
| CF | 26/49 (53) | 103/26 | 3.96 |
| ST | 5/19 (26) | 20/5 | 4.00 |
| CF HLY1 | 4/10 (40) | 17/4 | 4.25 |

[a] Totals do not include drug resistances to rifampin or cephalothin.

[b] Absence of all four plasmid-mediated characteristics.

One conclusion is that the more plasmid DNA a microorganism possesses, the greater is its chance of possessing drug resistance; and, since ETEC usually possess a number of plasmids, ETEC have a better than average chance of expressing drug resistance. A second conclusion is that ETEC diarrhea should not be treated with antibiotics because a positive selective pressure might be induced by an antibiotic which would not only fail to eliminate the ETEC but actually increase their number and prolong their normally short duration. ETEC diarrhea in adults is usually a self-limited disease entity requiring rehydration at most (31).

Six strains possessing the ST LT CF phenotypic pattern expressed no drug resistance. The significance of this finding is not apparent, but it is known that the ST gene(s) can insert into a CF plasmid (40). In the near future, LT gene(s) may also be found on a transposon (35). Theoretically, if a CF plasmid possessed one or a few insertion sites for commonly occurring transposons, this site or sites could be filled by the ST or LT genes or both. Then we could expect to find few or no sites remaining for antibiotic transposons. It is interesting (Table 4) that strains with the CF-only phenotypic pattern possessed an average of 3.96 resistances to antibiotics, whereas strains with CF ST and CF LT patterns possessed 2 and 1.4 different antibiotic resistances, respectively. As stated above, strains with the CF ST LT pattern showed no antibiotic resistances.

Further phenotypic and genotypic studies should also be carried out to determine whether there are associations between particular resistance patterns and particular plasmids. Should such associations exist, they may lead to a possible screening assay, based on antibiotic susceptibility testing, for the presence or absence of plasmids implicated in the pathogenicity of *E. coli*.

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