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Ureolytic *Escherichia coli* of Human Origin: Serological, Epidemiological, and Genetic Analysis

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Forty-five strains of ureolytic *Escherichia coli* of human origin, isolated in the United States between 1956 and 1977, were characterized by geographical distribution, site of infection, serotype, resistance to antibiotics, and biochemical reactions. All strains were studied for the ability to generate clones of nonureolytic *E. coli* (segregants), and a subset of these were selected for plasmid analysis and a variety of bacterial matings. There did not appear to be a common geographical distribution, serotype, antibiogram, or other aberrant biochemical reactions other than the hydrolysis of urea among these strains. The predominance of urinary tract isolates (46.7% total) may reflect a relationship between urea hydrolysis and pathogenesis at this site. Ten of the strains (22.2%) did segregate nonureolytic *E. coli* colonies, and all possessed at least one common plasmid species with a molecular weight of about 65×10^6. Only strain 1138-77 serotype O16:H6 conjugally transfered the ability to hydrolyze urea, ferment sucrose, and resist inhibition by sulfadiazine simultaneously. The resulting, recombination-deficient *E. coli* K-12 transconjugant was found to possess a plasmid with a molecular weight of about 80×10^6 to 90×10^6.

Ureolytic *Escherichia coli* are significant for several reasons. (i) Until recently the ability to hydrolyze urea was rarely documented in *E. coli*. In fact, this biochemical reaction is often used diagnostically in a screening set of biochemicals to exclude the presence of *E. coli*. (ii) From an evolutionary standpoint, the emergence or recognition of this trait in *E. coli* may elucidate the origin of a urease gene(s) in *E. coli* and its relationship to other ureolytic *Enterobacteriaceae*. (iii) The ability to use urea and produce alkaline conditions would appear to play a role in the pathogenesis at several sites of infection, especially in the urinary tract where alkaline conditions can cause renal damage, stone formation, and antibiotic inactivation by the following reaction (10-12):

$$NH_2\,CO\,NH_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_3 + H_2\,CO_3$$
$$2NH_3 + H_20 \longrightarrow NH_4 + OH$$

Independent observations that several organisms spontaneously lost the ability to use urea as a nitrogen source indicated the possible mediation of this characteristic by a plasmid-located gene(s) (3, 9, 27). In the investigation described here, we examine the hypothesis that urease activity in *E. coli* is mediated by plasmids.

MATERIALS AND METHODS

Bacterial strains. Fifty-seven ureolytic strains of *E. coli* were received by the Enteric Section, Center for Disease Control, Atlanta, Ga., for identification from 1956 through 1977. Forty-five of these, isolated in the United States from well-defined human sources, were used in this study (Table 1). Recipient *E. coli* K-12 strains used in bacterial matings were C600Nx (F^- *thr leu thi lacY gyrA* [formerly *nalA*], HB101 (F^- *leu pro lacY gal thi recA hsdR hsdM* endo I Sm^r), and 185Nx (F^- *nal*).

Media. Routine biochemical media and methods are described elsewhere (7). Urea hydrolysis was detected on Christensen urea agar (25). Appropriate positive and negative controls were run for each lot of biochemicals.

Serotyping. Serotyping was done in the Enteric Section, Center for Disease Control (7).

Segregation. Nonureolytic *E. coli* segregants were detected by isolating individual colonies from a ureolytic *E. coli* culture which had been plated on a nonselective medium (Trypticase soy agar [TSA], BBL Microbiology Systems), assaying from 5 to 10 of these isolated colonies on Christensen urea slants, and simultaneously subculturing to a numbered quadrant of a fresh TSA plate. Hydrolysis of urea and the resultant akaline environment prevented the routine recovery of ureolytic clones directly from plates of urea agar. Diffusion of this high pH metabolite also inhibited the recovery of nonureolytic *E. coli* clones. All segregants were tested for antibiotic susceptibility

and biochemical characteristics.

Antibiotic sensitivity testing. The high-potency, disk diffusion test of Bauer et al. (2) was routinely used to test susceptibility of organisms to nalidixic acid (NA), sulfadiazine (SD), chloramphenicol (CH), tetracycline (TC), streptomycin (SM), kanamycin (KM), gentamicin (GM), colistin (CO), penicillin (P), carbenicillin (CB), ampicillin (AM), cephalothin (CF) and trimethoprim (TMP). Minimal inhibitory concentrations (MICs) of selected antibiotics for selected strains were determined by standard methods.

Genetic transfer. Conjugation experiments were performed as described elsewhere (26) by using a MacConkey agar base (Difco Laboratories) with 1% lactose, raffinose, or inositol, and the following concentrations of selective and counterselective antibiotics: NA (20 μg/ml), KM (25 μg/ml), TC (10 μg/ml), and SD (350 μg/ml). Mating mixtures were incubated at 25 and 35°C for 2 to 24 h. They were then plated onto the appropriate medium and incubated overnight at 35°C; individual colonies were tested on urea agar. Antibiotic susceptibilities were determined on at least three transconjugants per plate.

Enterotoxin testing. *E. coli* strains and control cultures were inoculated into TSB and 0.6% yeast broth from a fresh agar slant, incubated with aeration overnight at 37°C, centrifuged, and filter sterilized. Filtrates were assayed in the Y-1 adrenal cell tissue culture system for heat-labile toxin and in the infant mouse assay for heat-stable toxin (5, 6).

Plasmid analysis. The plasmid deoxyribonucleic acid (DNA) of ureolytic *E. coli*, segregants, and transconjugants was analyzed by agarose gel electrophoresis of partially purified cell lysates from 40 ml of overnight broth cultures (18, 26). Gels were prepared and run as described by Meyers et al., and an estimated plasmid molecular weight was based on the migration distance in the gel of plasmids of known molecular weight (18).

Purified DNA was isolated from selected strains by centrifugation of cleared lysates in cesium chloride-ethidium bromide (CsCl-EtBr) gradients. Cleared lysates were derived from 100 ml of broth culture shaken overnight as described elsewhere (8). All gradients were centrifuged at 35,000 rpm at 10°C for 45 to 49 h. The plasmid DNA band was visualized by ultraviolet illumination and collected by puncture and gravity flow. EtBr was immediately extracted by using isopropanol saturated with CsCl and water. The plasmid DNA fraction was dialyzed overnight against dilute buffer, precipitated overnight at −10°C in two volumes of ethanol, and stored at −20°C.

Restriction endonucleases *Eco*RI, *Bam*I, and *Hin*dIII (Miles Laboratories) were reacted with 0.5 mg purified plasmid DNA and with lambda phage DNA as a control for molecular weights and complete digestion. Reaction mixtures for *Bam*I and *Hin*dIII enzymes contained 6 mM tris(hydroxymethyl)aminomethane, 50 mM NaCl, 6 mM $MgCl_2$ (pH 7.4), and 1.0 ml of enzyme in 50 ml, total volume. *Eco*RI reaction mixture contained 100 mM tris(hydroxymethyl)-aminomethane, 50 mM NaCl, 15 mM $MgCl_2$, and 5 mM ethylenediaminetetraacetic acid, pH 7.5. Reactions occurred at 37°C for 1 h and were terminated by incubation at 65°C for 5 min. Resulting fragments were analyzed by agarose gel electrophoresis.

RESULTS

The geographical distribution of laboratories sending cultures of ureolytic *E. coli* demonstrated no statistically significant pattern. Only five states sent more than two isolates from 1956 through 1977: Wisconsin, New Jersey, New York, California, and Colorado; each sent four isolates for identification or confirmation.

The distribution of isolates according to source of isolation is given in Table 1. The urinary tract was the most frequent site of infection; almost half of all strains were isolated from specimens from that location. The stool as a frequent source of *E. coli* strains was not surprising and accounted for ca. 31% of the isolates. Routine assays for *E. coli* heat-labile and heat-stable toxins were negative for the 14 stool cultures and 10 of the urinary tract cultures.

All ureolytic *E. coli* strains received before 1976 and several received after that date were serotyped. No serotype was common to these strains in general or when grouped by geographical source or antibiotic susceptibility. Table 2 describes the serotype, site of infection, source, and year of isolation for 27 of the 45 ureolytic *E. coli* strains listed in Table 1. Serotype O18a,c: H7 seems to occur most frequently among urine isolates, and O26 occurs most frequently among stool isolates of ureolytic *E. coli*.

Most of the ureolytic *E. coli* strains were sensitive to the set of antibiotics tested, with a predicted uniform resistance to PG and uniform susceptibility to GM, NA, and CO (Table 3). In addition to P, SD was the only other ineffective antibiotic; 17 strains, or 38% of all cultures tested, were resistant. This sulfaresistance was generally seen as bacterial growth up to the antibiotic disk corresponding to a high MIC (>3.5 μg/ml). These organisms were usually sensitive to TMP-sulfamethoxazole (Bactrim).

A relationship appeared to exist between the most frequent site of isolation (the urinary tract)

TABLE 1. *Source of 45 strains of ureolytic E. coli: site of infection*

Site	No.	%
Urinary tract	21	46.7
Stool	14	31.2
Blood	3	6.7
Wound	2	4.4
Throat	2	4.4
Cervix	1	2.2
Ear	1	2.2
Burn	1	2.2

TABLE 2. *Serotypes of 27 strains of ureolytic E. coli*

Site of infection	Serotype	Source/year
Urine	O6:NM[a]	North Carolina, 1972
	O7:H1	California, 1973
	O16:H6	Georgia, 1977
	O18a,c:H7	Illinois, 1971
	O18a,c:H7	New York, 1974
	O18a,c:H7	Tennessee, 1974
	O18a,c:H7	New Jersey, 1974
	O82:H31	Minnesota, 1970
	O132:NM	Indiana, 1972
	Ound:H4[b]	Washington, 1974
	Rough:H31	Maryland, 1971
	Rough:NM	California, 1976
Stool	O5:NM	California, 1974
	O26:NM	Michigan, 1973
	O26:NM	Colorado, 1973
	O26:H11	California, 1974
	O88:NM	Kansas, 1974
	O135:H11	Montana, 1973
	O135:NM	Colorado, 1975
	Ound:NM	Wisconsin, 1968
	Ound:H45	Tennessee, 1971
Other	O22:H1	Colorado, 1976
	O76:H21	Michigan, 1973
	O76:H21	Michigan, 1973
	O129:NM	North Carolina, 1974
	O157:H45	Pennsylvania, 1974
	Ound:H4	New York, 1972

[a] NM, nonmotile.

[b] Ound, O antigen unidentified with available antisera.

and resistance to SD. Of the 21 strains isolated from the urinary tract, 13 were resistant and 8 were sensitive to SD; of the 24 strains isolated from other sources, 4 were resistant and 20 were sensitive to SD. Statistically, this relationship was significant at a level of $P < 0.002$ (Fisher exact probability). We had insufficient data in our files to consider antibiotic therapy before or after culturing.

Biochemically, there were several isolated, unusual reactions for *E. coli* in general, but none of these was common to all or most ureolytic *E. coli* strains. Fermentation reactions for commonly tested carbohydrates are given in Table 4. A comparison with typical *E. coli* reactions listed in the last column showed a close correlation with the actual percentages generated by the ureolytic *E. coli* strains.

Six of the 45 ureolytic *E. coli* strains lost the ability to hydrolyze urea during storage on blood agar base slants and stabs with paraffin-corked tubes at room temperature. A detailed description of numbers of segregants is given in Table 5. Including the six cultures mentioned above, 22.2% of the strains spontaneously lost the ability to hydrolyze urea at an overall frequency of ca. 5% for individual isolates.

Biochemical characterization and antibiotic tests on five parent-segregant sets of cultures were studied in detail. These data and the original source of each culture are given in Table 6. The five strains were chosen on the basis of their ability to generate segregants and without prior knowledge of any other strain characteristics. Four of the five strains were urinary tract isolates, and all were resistant to SD. All segregants of 1138-77 and 2521-74 were sensitive to SD, and one segregant of 708-74 lost resistance to KM along with its ability to hydrolyze urea. Both 1138-77 and 708-74 lost the ability to ferment sucrose, and 708-74 also lost the ability to ferment raffinose in all nonureolytic *E. coli* segrants.

The five parent strains reported above were mated at 25 and 37°C with *E. coli* K-12 strains C600Nx (*lac*$^-$, NA) and HB101 (*lac*$^-$, SM), with counterselection by SD, TC, and KM being used where indicated. Only strain 1138-77 was able to transfer ureolysis along with resistance to SD at

TABLE 3. *Antibiotic susceptibility of ureolytic E. coli*

Antibiotic[a]	Resistant	Sensitive	Resistance (%)
NA	0	45	0
SD	17	28	38
CH	0	45	0
TC	5	40	12
SM	6	39	15
KM	6	39	15
GM	0	45	0
CO	0	45	0
P	45	0	100
CB	1	44	2.3
AM	4	41	9.8
CF	1	44	2.3

[a] Criteria for determining resistance and sensitivity are those of Bauer et al. (2).

TABLE 4. *Substrate utilization by ureolytic E. coli*

Substrate	Total no.	No. positive	% Positive	Typical *E. coli* % (7)
Lactose	27	22	81	91.6
Sucrose	27	10	37	53.7
Raffinose	27	12	44	49.4
Dulcitol	27	20	77	49.3
Salicin	27	12	44	36
Cellobiose	27	1	4	3.7
Adonitol	27	1	4	5.2
L-Rhamnose	27	23	86	83.5
D-Sorbitol	27	24	89	80.3
i-Inositol	32	1	3	0.9

a detectable frequency (10^{-5}) after overnight mating at 37°C. Ureolytic transconjugant HB101 was mated with *E. coli* K-12 strain 185Nx (*lac*$^+$ NA). Second generation transconjugants were again detected (10^{-4}) after overnight mating at 37°C. These strains are compared in Table 7.

An initial plasmid analysis performed by electrophoresing the crude lysates of 15 ureolytic *E. coli* strains indicated that all strains contained at least one species of a large-molecular weight extrachromosomal DNA of ca. 65×10^6 by agarose gel electrophoresis. The five parent-segregant groups were further examined by using CsCl-EtBr gradient preparations of plasmid DNA (Fig. 1). Another comparison of strain 1138-77 and ureolytic transconjugants is presented in Fig. 2. It is apparent that the newly acquired plasmid DNA band in *E. coli* K-12 strains HB101, C600 Nx, and 185 Nx (which are normally free of any plasmid DNA) is larger than the 65×10^6-dalton band in parental ureolytic strains. The band, tentatively designated pKW5, appears to be $>80 \times 10^6$ daltons.

TABLE 5. *Segregation of nonureolytic E. coli clones from ureolytic E. coli strains*

Strain description	No.	Total tested	%
Ureolytic, after storage	39	45	86.7
Segregant, nonureolytic clones	23	431	5.3
Generators of segregants	10	45	22.2

DISCUSSION

It has become increasingly evident that many of the genes controlling substrate utilization, metabolic end products, antibiotic resistance, and virulence factors are located in plasmids (14, 19, 20, 23-25). As a self-replicating extrachromosomal genetic element that is stably inherited, the plasmid DNA is maintained in a host cell without specific selection. Many plasmids

TABLE 6. *Characterization of ureolytic E. coli parent strains and nonureolytic E. coli segregants (seg)*

Strain	Serotype	Site	Biochemicals[a]					Antibiotic susceptibility[b]		
			Urea	Lac	Suc	Raf	Hly	SD	TC	KM
1138-77	O6:H16	Urine	+	+	+	−	+	R	S	S
1138-77, seg			−	+	−	−	+	S	S	S
708-74	O157:H45	Blood	+	+	+	+	−	R	S	R
708-74, seg 1			−	+	−	−	−	R	S	S
708-74, seg 2			−	+	−	−	−	R	S	R
651-73	O7:H1	Urine	+	+	−	−	−	R	R	S
651-73, seg			−	+	−	−	−	R	R	S
4448-74	O18a,c:H7	Urine	+	+	−	−	+	R	S	S
4448-74, seg			−	+	−	−	+	R	S	S
2521-74	O18a,c:H7	Urine	+	+	−	−	−	R	S	S
2521-74, seg			−	+	−	−	−	S	S	S

[a] Lac, Lactose fermentation; Suc, sucrose fermentation; Raf, raffinose fermentation; Hly, hemolysis of sheep erythrocytes in agar base.
[b] R, Resistant; S, susceptible.

TABLE 7. *Ureolytic transconjugants from ureolytic E. coli parent strain 1138-77*

Strain	Urea	Biochemicals[a]			Antibiotic[b] susceptibility		
		Lac	Suc	Raf	SD	NA	SM
1138-77	+	+	+	−	R	S	S
1138-77 segregant	−	+	−	−	S	S	S
HB101 recipient	−	−	−	−	S	S	R
C600Nx recipient	−	−	−	−	S	R	S
HB101 (1138) transconjugant	+	−	+	−	R	S	R
C600Nx (1138) transconjugant	+	−	+	−	R	R	S
185Nx recipient	−	+	−	−	S	R	S
185Nx (HB101 × 1138) transconjugant	+	+	+	−	R	R	S

[a] Lac, Lactose fermentation; Suc, sucrose fermentation; Raf, raffinose fermentation; Hly, hemolysis of sheep erythrocytes in agar base.
[b] R, Resistant; S, susceptible.

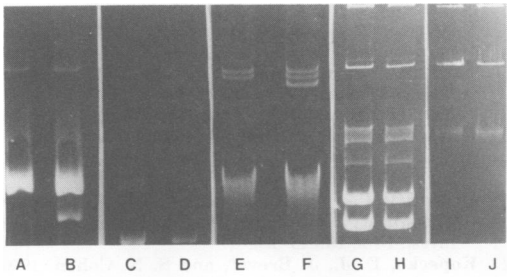

FIG. 1. *Agarose gel electrophoresis of purified plasmid DNA extracted from ureolytic E. coli parent strains and nonureolytic segregants. (A) 2521-74; (B) segregant 2521-74; (C) 1138-77; (D) segregant 1138-77; (E) 4448-74; (F) segregant 4448-74; (G) 708-74; (H) segregant-2 708-74; (I) 651-73; (J) segregant 651-73.*

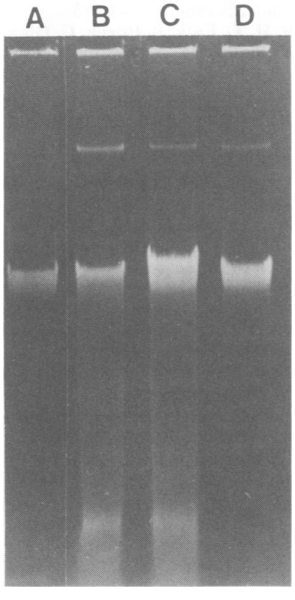

FIG. 2. *Agarose gel electrophoresis of plasmid DNA from crude lysates of: (A) ureolytic E. coli strain 1138-77; (B) ureolytic transconjugant C600Nx; (C) ureolytic transcojugant HB101; (D) ureolytic second-generation transconjugant 185Nx.*

also contain determinants for conjugation promotion and transfer of genetic material to other bacterial hosts. In this way, genes located on plasmid vehicles may be spread throughout the microflora of the human gut or, possibly, of the urinary tract when conditions and selective pressures exist.

As recently reported for genetic loci determining the fermentation of lactose (4), genes may reside in transposable segments of DNA which spontaneously insert or delete into host plasmid or chromosomal DNA without classical recombination (13). All of these discoveries have led to the recognition that variation within many genera and species is probably mediated by plasmids. Hydrolysis of urea by *E. coli* is usually considered a rare event (7) similar to but apparently much less frequent than H_2S production by *E. coli*. Because H_2S production and the fermentation of various carbohydrates by *E. coli* have been shown to be plasmid mediated, these infrequently isolated ureolytic *E. coli* are good candidates for genetic studies (14).

Ureolytic bacteria have the potential for ammonia production and subsequent pH increases which may affect tissues, competing microflora, and the action of antibiotics at a variety of sites (12, 16, 17). In fact, intestinal flora may generate ammonia which is absorbed into the circulation and implicated in hepatic failure and coma (21). More commonly, the pathogenesis of a variety of urease-producing organisms, especially *Providencia rettgeri, Proteus mirabilis,* and *Staphylococcus saprophyticus*, for the urinary tract has been well documented (1, 17, 22). Further, in a recent report of ureolytic *E. coli*, seven of eight isolates were from urinary tract infections (27). These data confirm our observations and implications that ureolytic *E. coli* is a pathogenic organism with a prediliction for the urogenital site.

The concomitant transfer of urea utilization, sucrose fermentation, and SD resistance indicates that the genetic determinants for these traits are located on the large molecular weight plasmid described above. Although sucrose fermentation has been previously transferred from *E. coli*, it occurred in only 1.9% of all isolates (24). The loss of urease production has been reported for several bacteria (3, 9), but no transfer or plasmid analysis has been demonstrated. Before our isolation of pKW5 there has been speculation that the urease gene(s) might be located on a nontransmissible plasmid (3, 9). The availability of strains with SD resistance permitted the mating and counterselection of a large number of strains, but transfer occurred with only one, 1138-77.

These conflicting observations may indicate that the urease gene(s) is located on more than one type of plasmid and possibly in close association with sucrose or raffinose genes in at least two ureolytic *E. coli* strains (1138-77 and 708-74). Genes determining the porcine enteric colonization factor K-88 have been observed in a similar relationship with genes for raffinose utilization (*raf*) (23). K-88 is apparently located on a transposable element in a plasmid also containing *raf* genes, and the transconjugants for K-88 and *raf* contain a higher molecular weight

plasmid than was known in the donor strain (23). Here, as there, this may mean that a recombination event is required to mobilize the urea plasmid. Further studies are needed to compare parent-transconjugant plasmids and the mechanisms of transfer. The postulation of a transposable urease gene seems valid in the light of the K-88 study and this report, but the apparent similarity of plasmid profiles between ureolytic *E. coli* and parent ureolytic *E. coli*-segregant nonureolytic *E. coli* strains argues against this mechanism.

The convenient presence of SD resistance in ureolytic *E. coli* from the human urinary tract may indicate more than a fortuitous event. There may be an association of antibiotic therapy for patients with infections at this site and selection of resistance as well as exchange of genetic material between organisms. In vitro R plasmid transfer has been reported between *Serratia* and *E. coli* in urine (20), and the possible acquisition of mobilizing or conjugation-encoding plasmids certainly exists. There are plausible mechanisms by which *E. coli* in urine could spread to the environment or gut under appropriate conditions. More detailed studies of other urease-producing *Enterobacteriaceae*, especially those found in the urinary tract, may better explain the possible selective advantages and the genesis of ureolytic *E. coli*.

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