APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1984, p. 930–935
0099-2240/84/110930-06$02.00/0

Vol. 48, No. 5

Resistance to Drugs and Heavy Metals, Colicin Production, and Biochemical Characteristics of Selected Bovine and Porcine *Escherichia coli* Strains†

NORMA M. HARNETT* AND CARLTON L. GYLES

Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Received 16 January 1984/Accepted 15 August 1984

A study was made of resistance to heavy metals and antibiotics, biochemical characteristics, and colicinogeny in selected strains of *Escherichia coli* of O serogroups 8, 9, 20, 64, 101, and X46. Of 42 strains that were investigated, 26 were porcine enterotoxigenic *E. coli* (ETEC), 8 were porcine non-enterotoxigenic *E. coli* (NETEC), and 8 were bovine ETEC. Multiple resistance to antimicrobial agents was common among the strains, and resistance to chloramphenicol and kanamycin was less common than resistance to other drugs, possibly reflecting the lower frequency of use of these agents in pigs and calves. Colicin production was a more common property of porcine ETEC (80.8%) than of porcine NETEC (25%), and all porcine ETEC of O serogroups 101 and 64 were colicinogenic. Equal numbers of bovine ETEC strains were colicinogenic as were non-colicinogenic. Resistance of bovine and porcine strains to sodium arsenate, mercury, and tellerium was 90, 16, and 5%, respectively. There was a close relationship between serogroup and biochemical reactions among the *E. coli* strains tested.

Several studies (2, 10, 13, 14, 18, 30, 43) have related biochemical properties of strains of *Escherichia coli* to serogroup and to pathogenicity of the organisms. Studies involving porcine strains have been conducted mainly on enterotoxigenic *E. coli* (ETEC) of "classical" serogroups (10, 33) and have demonstrated a relationship between patterns of biochemical reactions and O serogroups (10, 14, 30). Biochemical characterization of bovine ETEC has demonstrated that six common O:K serogroups could be separated into five biotypes and that biotype was related to serogroup (2).

A number of these investigations have paid particular attention to the relationship between colicinogenicity and pathogenicity (14, 43). The study by Vasenius (43) on colicinogenic properties of *E. coli* belonging to classical serogroups revealed that 60% of strains isolated from porcine colibacillosis were colicinogenic compared with 17% from healthy pigs. Larsen (14) found colicin production to be related to serological type and to occur most often among strains of serogroup O149:K91. The incidence of colicinogenicity among bovine strains ranged from 32 to 61%, and pathogenic strains were more frequently colicin producers than were nonpathogenic strains (23, 44).

Strains of *E. coli* are frequently resistant to heavy-metal ions (4, 5, 24, 42), and the resistances can often be transferred by conjugation. Plasmid-mediated resistance to Hg, Co, and Ni (28), arsenate (12, 36), arsenite (29), and copper (41) has been observed in *E. coli*. Some of these metals have been used in animal feeds for growth promotion or therapy (1, 37); others are known or suspected as causes of environmental pollution (3, 20, 24). In some instances resistance to these metals is mediated by the same plasmid that determines resistance to antibiotics (20, 21). There are also reports of single plasmids in *E. coli* which bear genes for combinations of properties such as colicin production, enterotoxigenicity, and biochemical reactions (17, 35; Harnett and Gyles, Can. J. Comp. Med., in press).

This study was designed to determine certain biochemical properties including colicinogenicity and resistance to antimicrobial drugs and heavy metals among bovine and porcine *E. coli* of serogroups characteristic of porcine class 2 ETEC and to provide information on association of patterns of biochemical properties with the different groups of *E. coli*.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* used are described in Tables 1 and 2. These strains have been examined previously for production of heat-labile enterotoxin (LT), heat-stable enterotoxins (ST), and colonization pili (11; N. M. Harnett, Ph.D. thesis, University of Guelph, Guelph, Ontario, 1982). The *E. coli* K-12 derivatives 711 and C600 were from our laboratory collection. *E. coli* RG192 with no plasmids and with plasmids R40-a, R478, and R45 served as controls for resistance to mercury (Hg), tellurite (Te), and arsenate (As). The *E. coli* K-12 strains ROW and 711 were used as indicator strains in colicin experiments.

Chemicals. Sodium arsenate, sodium arsenite, lead acetate, lead nitrate, cadmium chloride, cobalt chloride, and cupric sulfate were obtained from Fisher Scientific Co.; zinc sulfate, silver nitrate, and mercuric chloride were from BDH; and potassium tellurite was from Matheson, Coleman & Bell.

Colicinogeny assay. Colicin production was detected by the agar overlay method (8). Cells were inoculated with sterile toothpicks onto LB medium (19) solidified with 1.5% agar and containing 5×10^{-3} M $CaCl_2$ (LTC plates). After overnight growth at 37°C, the cells were killed by exposing the plates to chloroform vapor for 30 min, and the plates were overlaid with LTC soft agar (0.6% agar) containing ca. 5×10^7 cells of *E. coli* K-12 ROW. The soft agar overlay was prepared by suspending 0.1 ml of an overnight culture grown in brain heart infusion broth (Difco Laboratories) in 2.5 ml of soft LTC agar kept at 45°C. Zones of inhibition were observed after overnight incubation at 37°C. The tests were duplicated with *E. coli* 711 as a colicin indicator strain. Strains which inhibited *E. coli* ROW or 711 were considered

* Corresponding author.

† This report represents a portion of a thesis submitted by N. M. Harnett to the Faculty of Graduate Studies, University of Guelph, as partial fulfillment of the requirements for the Ph.D. degree.

TABLE 1. Strains of porcine *E. coli* of O serogroups 9, 20, 64, and 101

Strain	Serogroup	Source[a]
G24[b]	O9:K103	P. A. M. Guinée
G46	O9:K55	P. A. M. Guinée
G47	O9:K2347	P. A. M. Guinée
G53	O20:K?	P. A. M. Guinée
G57	O101:K28:K99	P. A. M. Guinée
G58[b]	O101:K28	P. A. M. Guinée
G59[b]	O101:K30	P. A. M. Guinée
G63	OX46:K103:987P$^+$	P. A. M. Guinée
M431	O101:K30:K99	H. W. Moon
M613	O101:K30:K99	H. W. Moon
M637	O64:K?:K99	H. W. Moon
P16[c]	O9:K103	H. W. Smith
P16M	O9:K103:987P$^+$	H. W. Smith
W482	O64:K?:K99	M. R. Wilson
W549	O101:K30	M. R. Wilson
W592[b]	O101:K30	M. R. Wilson
W2920A	O9:K103	M. R. Wilson
W2948[b]	O101:K30	M. R. Wilson
W2954[b]	O101:K30	M. R. Wilson
W3013	O64:K?:K99	M. R. Wilson
W3027	O9:K103:987P$^+$	M. R. Wilson
T211	O101:K30:K99	E. M. Kohler
T311	O9:K35:K99	E. M. Kohler
0203	O20ab:K?	E. M. Kohler
1003	O20ab:K?	E. M. Kohler
0329-A	O9:K103:K88ac	E. M. Kohler
1104	O9:K35	E. M. Kohler
0926-B-9C	O9:K35	E. M. Kohler
1129S	O101:K30:K99	E. M. Kohler
1216	O101:K30:K99	E. M. Kohler
0919(W)[b]	O101:K30	E. M. Kohler
0919(F)[b]	O101:K30	E. M. Kohler
1129R	O101:K$^-$:K99	E. M. Kohler
1123	O101:K$^-$:K99	E. M. Kohler

[a] P. A. M. Guinée, National Institute for Public Health, Bilthoven, The Netherlands; H. W. Moon, National Animal Disease Laboratory, Ames, Iowa; H. W. Smith, Houghton Poultry Research Station, Houghton, Huntingdon, Great Britain; M. R. Wilson, Ontario Veterinary College, Guelph; E. M. Kohler, Ohio Agricultural Research and Development Center, Wooster.

[b] These strains did not produce STa, STb, or heat-labile enterotoxin (NETEC).

[c] Strain P16 is a spontaneous mutant of P16M which has lost the ability to produce STa and 987P pili.

to be colicin producers. The two colicin indicator strains, ROW and 711, were shown to be susceptible to the standard set of colicins (8).

Determination of resistance to antimicrobial agents. Resistance to antibiotics was determined by the agar dilution method with a Steers replicator (34). The bacterial culture was grown at 37°C in Penassay Broth (Difco), diluted 1,000-fold in 0.2 M sodium phosphate buffer (pH 7.2), and inoculated (5 μl) onto DST agar (Oxoid Ltd.) or MacConkey agar (Difco) containing the appropriate antibiotic. For the determination of susceptibility to sulfamethoxazole, 1% lysed horse blood was added to DST agar. The inoculated plates were incubated for 16 to 20 h at 37°C before the tests were read. Concentrations (micrograms per milliliter) of antibiotics used to discriminate between resistance and susceptibility were as follows: ampicillin, 8; chloramphenicol, 16; kanamycin, 8; streptomycin, 10; sulfamethoxazole, 10; tetracycline, 4.

MICs were determined on DST agar.

TABLE 2. Strains of bovine ETEC

Strain	Serogroup	Source[a]
483	O9:K35:K99	L. L. Myers
490	O101:K30:K99	L. L. Myers
505	O101:K28:K99	L. L. Myers
524	O8:K25:K99	L. L. Myers
559	O8:K28:K99	L. L. Myers
W1-1	O20:K?:K99	L. L. Myers
B41	O101:K30:K99	H. W. Smith
B44	O9:K30:K99	H. W. Smith

[a] L. L. Myers, Montana State University, Bozeman; H. W. Smith, Houghton Poultry Research Station.

Determination of resistance to heavy metals. To determine heavy-metal resistance, quantitative susceptibility tests were performed by replica plating 1:100 dilutions of overnight Mueller-Hinton (MH) broth cultures of *E. coli* strains onto MH agar containing graded concentrations of the metal inhibitor. MICs were recorded as the lowest concentration of metal which prevented growth after overnight incubation at 37°C. The following millimolar concentrations of metal salts were used: $AgNo_3$, 2.5, 5, 10, 20; Na_2HAsO_4, 12.5, 25, 50, 100, 200; $NaAsO_2$, 0.1, 1.0, 10, 100, 200; $CdCl_2$, 0.01, 10; $CoCl_2$, 2, 5, 10, 20; $CuSO_4$, 4, 5, 10, 20; $HgCl_2$, 0.1, 1.0; $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$, 1, 10, 20; K_2TeO_3, 0.02, 0.1; $ZnSO_4$, 0.01, 0.1, 1.0, 10. Stock solutions of the metal salts were made in distilled water, sterilized by membrane filtration (pore size, 0.45 μm; Millipore Corp.), and kept at 4°C for no longer than 7 days. Copper-sulfate media were prepared by adding appropriate amounts of stock solution to MH agar, adjusting the pH to 7.4 with NaOH, and autoclaving the solution at 121°C for 15 min (41). Negative and positive control strains were tested.

Biochemical tests. Decarboxylase tests were conducted by the method of Falkow (7). Arginine, ornithine, and lysine decarboxylase media (Difco) were inoculated with organisms from an 18-h-old culture and incubated for 4 days at 37°C. Sterile paraffin (2 ml) was added to each tube after inoculation. Basal medium without the amino acids served as the negative control. The decarboxylase tests were read as positive when the medium became alkaline (purple) within 4 days and the negative control remained acid (yellow).

Tests were conducted to determine fermentation of the following carbohydrates: adonitol, arabinose, dextrin, dulcitol, galactose, inulin, inositol, lactose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. Phenol red broth (Difco) was used as the basal medium with phenol red as the indicator. The carbohydrate

TABLE 3. Colicin production and resistance to sodium arsenate and mercuric chloride among strains of bovine ETEC

Strain	Serogroup	Resistance to final concn (mM) of[a]:						Colicin production[a]
		As ($NaHAsO_4$)					Hg ($HgCl_2$)	
		12.5	25	50	100	200	0.1	
B41	O101:K30:K99	+	+	+	+	+	+	+
490	O101:K30:K99	+	+	+	+	−	+	−
483	O9:K35:K99	+	+	+	+	−	−	−
505	O101:K28:K99	+	+	+	−	−	+	−
559	O8:K25:K99	+	+	+	−	−	−	−
524	O8:K85:K99	+	+	−	−	−	+	+
W1-1	O20:K?:K99	+	+	−	−	−	−	+
B44	O9:K30:K99	−	−	−	−	−	−	+

[a] +, Positive; −, negative.

TABLE 4. Colicin production and resistance to sodium arsenate and mercuric chloride among strains of porcine *E. coli*

Strain	Serogroup	Resistance to final concn (mM) of[a]:						Colicin production[a]
		As ($NaHAsO_4$)					Hg ($HgCl_2$)	
		12.5	25	50	100	200	0.1	
G24	O9:K103	−	−	−	−	−	−	−
G46	O9:K55	+	+	−	−	−	−	−
G47	O9:K2347	+	−	−	−	−	−	−
G53	O20:K?	+	+	−	−	−	−	−
G57	O101:K28:K99	+	+	−	−	−	−	+
G58	O101:K28	+	+	−	−	−	−	−
G59	O101:K30	+	+	−	−	−	−	+
G63	OX46:K103:987P$^+$	+	+	+	+	−	−	+
M431	O101:K30:K99	+	+	+	−	−	−	+
M613	O101:K30:K99	+	+	+	+	−	−	+
M637	O64:K?:K99	+	+	−	−	−	−	+
W482	O64:K?:K99	+	+	+	+	−	−	+
W549	O101:K30	+	+	+	+	−	+	+
W592	O101:K30	+	+	+	−	−	−	+
W2920A	O9:K103	+	+	+	+	−	−	+
W2948	O101:K30	+	+	+	+	−	+	−
W2954	O101:K30	+	+	+	−	−	+	−
W3013	O64:K?:K99	+	+	+	+	−	−	+
W3027	O9:K103:987P$^+$	+	+	+	+	−	−	+
P16	O9:K103	+	+	+	−	−	−	−
P16M	O9:K103:987P$^+$	+	+	+	−	−	−	+
T211	O101:K30:K99	+	+	+	+	+	−	+
T311	O9:K35:K99	+	+	+	+	+	+	−
0203	O20ab:K?	+	+	+	+	−	−	+
1003	O20ab:K?	+	+	+	+	+	−	+
0329-A	O9:K103:K88ac	+	+	+	+	−	−	+
1014	O9:K35	+	+	+	+	−	−	+
0926-B-9C	O9:K35	+	+	+	+	−	−	+
1129S	O101:K30	+	+	+	+	+	−	+
1216	O101:K30:K99	+	+	+	+	+	−	+
0919(W)	O101:K30	−	−	−	−	−	−	−
0919(F)	O101:K30	−	−	−	−	−	−	−
1129R	O101:K$^-$:K99	+	+	+	+	−	−	+
1123	O101:K$^-$:K99	+	+	+	+	−	+	+

[a] +, Positive; −, negative.

was added to a final concentration of 0.5%. The tubes were inspected daily for 7 days. An inverted Durham tube was used for detecting gas formation.

Triple sugar iron agar (Difco) was used for detection of H_2S production, and Christensen urea agar (Difco) was inoculated to determine production of urease. Hemolysis was observed on 5% calf blood agar plates.

Experiments. All 34 isolates of bovine and porcine ETEC and the 8 porcine non-enterotoxigenic *E. coli* (NETEC) strains were tested for production of colicin, for susceptibility to antimicrobial drugs, and for resistance to nine heavy metals. The strains were also examined for their ability to ferment 17 different carbohydrates, to produce hemolysin, urease, and H_2S, and to decarboxylate lysine, ornithine, and arginine.

RESULTS

Production of colicin(s) by the *E. coli* strains. The results of the tests for production of colicin(s) by the *E. coli* strains are shown in Tables 3 and 4 and summarized in Table 5. Identical results were obtained with *E. coli* strains ROW and 711 as colicin indicator strains. Over 80% of the porcine ETEC strains produced colicin, whereas only 25% of the porcine NETEC strains were positive for colicin production. The numbers of bovine strains that were positive for production of colicin were the same as the numbers that were negative.

Resistance to heavy metals. All the bovine ETEC strains, except strain B44, showed resistance to sodium arsenate at the concentrations tested (Table 3). There was variability in the level of resistance, with strains B41, 490, and 483 resistant to the highest level of arsenate. Bovine strains, except strain B44, were resistant to sodium arsenite at a level of 1 mM. Mercury resistance was present in 50% of the bovine ETEC strains (Table 3). The MIC of mercury for strains B41, 524, 490, and 505 was greater than 0.1 mM. Bovine strains were also resistant to cadmium chloride, zinc sulfate, lead acetate, and lead nitrate at concentrations of 1 mM but sensitive at concentrations of 10 mM. For cobalt chloride, all bovine strains were resistant to 2 mM and sensitive to 5 mM. MICs of copper sulfate for all bovine strains were >10 mM.

None of the bovine ETEC strains was resistant to silver nitrate or potassium tellurite.

TABLE 5. Production of colicin by the *E. coli* isolates

Type of strains	No. of strains tested	No. colicin positive (%)
Bovine ETEC	8	4 (50)
Porcine ETEC	26	21 (80.8)
Porcine NETEC	8	2 (25)

A total of 91% of the porcine strains of *E. coli* were resistant to sodium arsenate (Table 4). A high percentage of the strains were resistant to 50 mM or greater concentrations of $NaHAsO_4$, whereas a few strains, especially those received from the Netherlands (all strains with the prefix G), were resistant to lower levels. All but three porcine strains (G24, 0919F, and 0919W) demonstrated resistance to $NaAsO_2$ at a level of 1 mM but were inhibited at 10 mM. In contrast, only 16% of the strains were resistant to mercury, and only two strains (G46 and 1014) were resistant to potassium tellurite. Porcine *E. coli* strains were also resistant to Zn and Pb at concentrations of 1 mM but sensitive at concentrations of 10 mM. For $CdCl_2$, all 34 strains were resistant at a concentration of 0.1 mM, 24 of 34 strains showed resistance in the form of confluent growth consisting of very fine colonies at a concentration of 1 mM, and all 34 strains were sensitive at a concentration of 10 mM. All porcine strains grew on medium containing $CoCl_2$ at a concentration of 2 mM but not at 5 mM.

The MIC of $CuSO_4$ for all porcine strains was 10 mM. None of the porcine strains were resistant to $AgNO_3$.

Resistance to antibiotics. Multiple drug resistance was demonstrated for most isolates. All but 3 strains were resistant to at least three drugs and 13 were resistant to five or more drugs. The predominant patterns of drug resistance were resistance to streptomycin, sulphonamide, and tetracycline and resistance to these drugs plus ampicillin and kanamycin. Table 6 shows the number of strains resistant to each of the six antibiotics. The only drugs to which the majority of the strains were susceptible were chloramphenicol and kanamycin.

Biochemical tests. All 42 strains of *E. coli* fermented lactose, galactose, mannose, mannitol, arabinose, trehalose, and xylose. All strains, except 1014 and 0926-B-9C, both of serogroup O9:K35, fermented sorbitol. None of the isolates fermented inositol, inulin, dextrin, or dulcitol, and only three strains, 524 (O8:K85), 559 (O8:K25), and W592 (O101:K30), fermented sucrose. The strains which fermented sucrose also fermented raffinose. All the strains were negative for production of H_2S, urease, and hemolysin. All strains, except G24 and 483, decarboxylated arginine. Two predominant patterns of decarboxylation were evident: strains either decarboxylated arginine, lysine, and ornithine or arginine and lysine.

DISCUSSION

Previous studies demonstrated that colicin production is more common among porcine class 1 ETEC than among porcine NETEC (23, 43). The present study reports data for *E. coli* of serogroups associated with porcine class 2 ETEC. All the porcine ETEC strains of serogroups 101 and 64 produced colicin, and among strains of serogroup O101:K30, six of seven ETEC strains but only two of six NETEC strains were colicin positive.

Strain P16 (O9:K103), which has lost the ability to produce STa and 987P pili, was colicin negative, although the parent strain was colicin positive. The locus of the genes for 987P pili has not been determined, and it is possible that all three properties are on a single plasmid. In a study of plasmids in *E. coli* of these serogroups, genes for Col, ST, and pili were frequently found on a single plasmid (Harnett and Gyles, in press; N. M. Harnett, Ph.D. thesis). It is also interesting that all strains with the 987P pilus antigen and all wild ETEC strains of serogroup O9:K103 were colicin positive and that the only NETEC strain of serogroup O9:K103, G24, was colicin negative. Furthermore, strain G24 was previously found to be enterotoxigenic (9) and has probably lost the genes for ST. Further investigations may yield useful results concerning the relationship among the genes for these properties.

The high frequency of multiple drug resistance among these isolates is consistent with earlier reports (10, 27, 30, 31). The lower frequency of resistance to chloramphenicol and kanamycin coincides with the lower frequency of use of these drugs in calves and pigs. Chloramphenicol resistance was detected only in recent isolates from Canada and the United States.

There was a relationship between serogroup and fermentation patterns, with small differences among strains of the same serogroup. These observations are similar to those made by Braaten and Myers (2) for calf strains. Fermentation and decarboxylation reactions permitted separation of the strains into eight groups which were related to serogroup (Table 7). Adonitol fermentation was seen mainly in strains of O serogroup 101, and all 10 ETEC strains and 6 of 9 NETEC strains of this O serogroup were positive. Braaten and Myers (2) found that only 2 of 52 bovine NETEC strains fermented adonitol and suggested that adonitol fermentation could be used as an indicator of pathogenicity among serogroup O101 *E. coli* from calves.

None of the strains produced hemolysin, H_2S, or urease. Hemolysis is a common feature of class 1 porcine ETEC strains (10, 30, 32, 39) but is only infrequently associated with other ETEC strains (2, 6, 26). Some strains of porcine ETEC have been shown to produce H_2S (22). The property is plasmid determined and the genes may be associated with those for tetracycline resistance (38) or for raffinose fermentation (15, 22). Urease production has been reported among certain class 1 ETEC strains from weaned pigs (14, 30).

Differences in biochemical reactions were not associated with the presence or absence of colonization pili on the strains. These findings are different from those reported by Isaacson and co-workers (13) for bovine strains.

Over 90% of the bovine and porcine strains showed resistance to arsenate (Tables 3 and 4). Arsenate resistance in strains of *E. coli* from animals has been shown to be transmissible (29), and plasmids with genes for arsenate resistance belong to incompatibility groups FII, N, and H_2 (37). There was "moderate resistance" (29) to arsenite in 38 of the 42 strains tested. Smith (29) reported that "moderate resistance" was present in 469 of 523 *E. coli* strains of animal origin, that a lower proportion of human *E. coli* isolates

TABLE 6. Antibiotic resistance of bovine and porcine *E. coli* to individual drugs

Sources of isolates	Total no. of strains	No. of isolates resistant to[a]:					
		Ap	Sm	Su	Tc	Km	Cm
Bovine	8	5	6	8	6	2	2
Porcine	34	18	25	31	26	11	2

[a] Symbols for drug resistances: Ap, ampicillin; Sm, streptomycin; Su, sulphonamides (sulfamethoxazole); Tc, tetracycline; Km, kanamycin; Cm, chloramphenicol.

TABLE 7. Relationship of serogroup to biochemical characteristics of bovine and porcine *E. coli*

Biochemical characteristic	No. of positive strains of serogroup:							
	O101 (n = 19)	O9[a] (n = 9)	O9:K35 (n = 4)	O64:K? (n = 3)	O20 (n = 4)	O8:K25 (n = 1)	O8:K85 (n = 1)	OX46 (n = 1)
Fermentation of:								
Adonitol	16	0	0	0	1	0	1	0
Raffinose	1	2	0	0	0	1	1	1
Salicin	18	0	2	3	3	1	1	1
Sucrose	1	0	0	0	0	1	1	0
Rhamnose	18	3	4	3	4	1	1	1
Decarboxylation of:								
Lysine	17	8	2	3	3	1	1	1
Ornithine	1	2	4	3	0	1	0	1

[a] Strains of O serogroup 9 other than those with K antigen 35.

showed resistance to arsenite, and that there was an association between serogroup and resistance.

Mercury resistance (Hg^r) was demonstrated for 50% of the bovine and 15.6% of the porcine strains. These findings are consistent with reports that Hg^r is common among isolates of *E. coli* (16, 25). Mercury resistance in *E. coli* is a plasmid-mediated property (25, 38) which has been associated with multiple drug resistance (20, 21). In one class 1 porcine ETEC strain the genes for Hg^r and for multiple drug resistance have been shown to be on a single plasmid (17). In one of the bovine ETEC strains in this study Hg^r and tetracycline resistance were consistently eliminated together.

Only 2 of the 42 *E. coli* strains were resistant to tellurite. Resistance to tellurite (Te^r) is an uncommon, plasmid-determined property among gram-negative bacteria and is associated with inhibition of coliphage development (40).

Despite the adjustment of the pH of MH broth after addition of $CuSO_4$ (41), differentiation between the test strains and the laboratory K-12 strains was not possible. The MICs of another four of the metals, Co, Zn, Pb, and Cd, also did not exceed the MICs of these metals for the *E. coli* K-12 strains and were at similar levels in both bovine and porcine strains.

None of the bovine and porcine strains in this study was resistant to silver nitrate. Silver resistance has been reported among hospital isolates (37) and in *E. coli* isolates from bay water (4).

The frequencies of antibiotic resistance and colicin production among the 42 isolates of bovine and porcine *E. coli* were high. The frequencies of resistance to arsenate, mercury, and tellerium were 90, 16, and 5%, respectively. In a recent report (Harnett and Gyles, in press), we demonstrated the cotransfer of antibiotic resistance determinants and production of ST, the K99 antigen, and colicin on a single plasmid. Further studies are in progress to identify the occurrence of heavy-metal resistance and other properties on plasmids in *E. coli* of these serogroups recovered from pigs and calves.

ACKNOWLEDGMENTS

We thank P. A. M. Guinée, E. M. Kohler, H. W. Moon, L. L. Myers, H. W. Smith, and M. R. Wilson for providing strains and Pat Yelf and Wyn Hall for typing the manuscript.

This work was supported by the Natural Sciences and Engineering Research Council, Canada.

LITERATURE CITED

1. **Barber, R. S., R. Braude, K. G. Mitchell, and J. Cassidy.** 1955. High copper mineral mixture for fattening pigs. Chem. Ind. (London) **74**:601.
2. **Braaten, B. A., and L. L. Myers.** 1977. Biochemical characteristics of enterotoxigenic and non-enterotoxigenic *Escherichia coli* isolated from calves with diarrhea. Am. J. Vet. Res. **38**:1989–1991.
3. **Carmody, D. J., J. B. Pearce, and W. E. Yasso.** 1973. Trace elements in sediments of New York Bight. Mar. Pollut. Bull. **4**:132–135.
4. **Chai, T.-J.** 1983. Characteristics of *Escherichia coli* grown in bay water as compared with rich medium. Appl. Environ. Microbiol. **45**:1316–1323.
5. **Elek, S. D., and L. Higney.** 1970. Resistogram typing – a new epidemiological tool: application to *Escherichia coli*. J. Med. Microbiol. **3**:103–110.
6. **Ellis, R. P.** 1978. Serologic and epidemiologic investigations of colibacillosis in pigs, p. 161–165. *In* S. Acres (ed.), Proceedings of the Second International Symposium on Neonatal Diarrhea. University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
7. **Falkow, S.** 1958. Activity of lysine decarboxylase as an aid in the identification of salmonellae and shigellae. Am. J. Clin. Pathol. **29**:598–600.
8. **Fredericq, P.** 1957. Colicins. Annu. Rev. Microbiol. **11**:7–22.
9. **Guinée, P. A. M., C. M. Agterberg, W. H. Jansen, and J. F. Frik.** 1977. Serological identification of pig enterotoxigenic *Escherichia coli* strains not belonging to the classical serotypes. Infect. Immun. **15**:549–555.
10. **Gyles, C. L., J. B. Stevens, and J. A. Craven.** 1971. A study of *Escherichia coli* strains isolated from pigs with gastro-intestinal disease. Can. J. Comp. Med. **35**:258–266.
11. **Harnett, N. M., and C. L. Gyles.** 1983. Enterotoxigenicity of bovine and porcine *Escherichia coli* of O groups 8, 9, 20, 64, 101 and X46. Am. J. Vet. Res. **44**:1210–1214.
12. **Hedges, R. W., and S. Baumberg.** 1973. Resistance to arsenic compounds conferred by a plasmid transmissible between strains of *Escherichia coli*. J. Bacteriol. **115**:459–460.
13. **Isaacson, R. E., H. W. Moon, and R. A. Schneider.** 1978. Distribution and virulence of *Escherichia coli* in the small intestine of calves with and without diarrhea. Am. J. Vet. Res. **39**:1750–1755.
14. **Larsen, J. L.** 1976. Differences between enteropathogenic *Escherichia coli* strains isolated from neonatal *E. coli* diarrhoea (N.C.D.) and post weaning diarrhoea (P.W.D.) in pigs. Nord. Veterinaermed. **28**:417–429.
15. **Magalhaes, M., and A. Veras.** 1977. Properties of pPE 1572 (Hys-Raf), a plasmid governing hydrogen sulphide production and raffinose fermentation in *Escherichia coli*. J. Gen. Microbiol. **99**:445–447.
16. **Marshall, B., S. Schluederberg, D. Rowse-Eagle, A. O. Summers, and S. B. Levy.** 1981. Ecology of antibiotics and heavy metal resistance in nature, p. 630. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity and ecology of bacterial plasmids. Plenum Publishing Corp., New York.
17. **Mazaitis, A. J., R. Maas, and W. K. Maas.** 1981. Structure of a naturally occurring plasmid with genes for enterotoxin produc-

tion and drug resistance. J. Bacteriol. **145**:97–105.
18. **Merson, M. H., F. Ørskov, I. Ørskov, R. B. Sack, I. Huq, and F. T. Koster.** 1979. Relationship between enterotoxin production and serotype in enterotoxigenic *Escherichia coli*. Infect. Immun. **23**:325–329.
19. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, H. Kozukue, and S. Mitsuhashi.** 1977. Mercury resistance and R plasmids in *Escherichia coli* isolated from clinical lesions in Japan. Antimicrob. Agents Chemother. **11**:999–1003.
21. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, H. Kozukue, and S. Silver.** 1977. Linkage of mercury, cadmium, and arsenate and drug resistance in clinical isolates of *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **33**:975–976.
22. **Ørskov, I., and F. Ørskov.** 1973. Plasmid-determined H_2S character in *Escherichia coli* and its relation to plasmid-carried raffinose fermentation and tetracycline characters: examination of 32 H_2S-positive strains isolated during the years 1950–1971. J. Gen. Microbiol. **25**:487–499.
23. **Papavassiliou, J.** 1962. Colicin producing and colicin sensitive types of 78 *E. coli* isolated from calves. Arch. Inst. Pasteur Hell. **8**:63–64.
24. **Porter, F. D., S. Silver, C. Ong, and H. Nakahara.** 1982. Selection for mercurial resistance in hospital settings. Antimicrob. Agents Chemother. **22**:852–858.
25. **Reis, M. H. L., M. J. C. Ramos, and L. R. Trabulsi.** 1978. Resistance to mercury in enteric organisms: frequency and genetic nature. Rev. Microbiol. **9**:24–29.
26. **Sherwood, D., D. R. Snodgrass, and G. H. K. Lawson.** 1983. Prevalence of enterotoxigenic *Escherichia coli* in calves in Scotland and Northern England. Vet. Rec. **113**:208–212.
27. **Sivaswamy, G., and C. L. Gyles.** 1976. Characterization of enterotoxigenic bovine *Escherichia coli*. Can. J. Comp. Med. **40**:247–251.
28. **Smith, D. H.** 1967. R factors mediate resistance to mercury, nickel and cobalt. Science **156**:1114–1116.
29. **Smith, H. W.** 1978. Arsenic resistance in enterobacteria: its transmission by conjugation and by phage. J. Gen. Microbiol. **109**:49–56.
30. **Soderlind, O.** 1971. Studies on *Escherichia coli* in pigs. I. Biochemical investigations. Zentralbl. Veterinaermed. Reihe B **18**:489–504.
31. **Sogaard, H.** 1973. Incidence of drug resistance and transmissible R factors in strains of *E. coli*. Acta Vet. Scand. **14**:381–391.
32. **Sojka, W. J.** 1965. *Escherichia coli* in domestic animals and poultry. Commonwealth Agricultural Bureau, Weybridge, England.
33. **Sojka, W. J., M. K. Lloyd, and E. J. Sweeney.** 1960. *Escherichia coli* serotypes associated with certain pig diseases. Res. Vet. Sci. **1**:17–27.
34. **Steers, E., L. Foltz, and B. S. Graves.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. **9**:307–311.
35. **Stoleru, G. H., G. R. Gerbaud, D. H. Bouanchaud, and L. LeMinor.** 1972. Etude d'un plasmide transferable determinant le production d'H_2S et la resistance à la tetracycline chez '*Escherichia coli*.' Ann Inst. Pasteur Paris **123**:743–754.
36. **Summers, A. O., and G. A. Jacoby.** 1977. Plasmid-determined resistance to tellerium compounds. J. Bacteriol. **129**:276–281.
37. **Summers, A. O., G. A. Jacoby, M. N. Swartz, G. McHugh, and L. Sutton.** 1978. Metal cation and oxyanion resistances in plasmids of gram-negative bacteria, p. 128–131. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
38. **Summers, A. O., and S. Silver.** 1972. Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. J. Bacteriol. **112**:1228–1236.
39. **Sweeney, E. J.** 1968. Haemolytic *Escherichia coli* in enteric disease of swine. Ir. Vet. J. **22**:10–15.
40. **Taylor, D. E., and A. O. Summers.** 1979. Association of tellerium resistance and bacteriophage inhibition conferred by R plasmids. J. Bacteriol. **137**:1430–1433.
41. **Tetaz, T. J., and R. K. J. Luke.** 1983. Plasmid-controlled resistance to copper in *Escherichia coli*. J. Bacteriol. **154**:1263–1268.
42. **Varma, M. M., W. A. Thomas, and C. Prasad.** 1976. Resistance to inorganic salts and antibiotics among sewage-borne *Enterobacteriaceae* and *Achromobacteriaceae*. J. Appl. Bacteriol. **41**:344–349.
43. **Vasenius, H.** 1967. The colicinogenic properties of porcine *Escherichia coli* strains. Acta Vet. Scand. **8**:195–200.
44. **Yadava, J. N. S., and B. M. Gupta.** 1971. Studies on colicinogeny, lysogeny and phage susceptibility of 153 strains of *E. coli* isolated from gastroenteritis of domestic animals. Indian J. Anim. Sci. **41**:428–433.