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Clonal Relationships among *Escherichia coli* Serogroup O78 Isolates from Human and Animal Infections

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We investigated the clonal relationships among 63 *Escherichia coli* strains of antigen serogroup O78 isolated from infections in humans, cattle, sheep, pigs, and chickens. Both septicemic and enterotoxigenic isolates were included in the study. A main group of 55 *E. coli* strains consisting of 52 septicemic isolates and 3 human enterotoxigenic *E. coli* isolates were clustered in related clones. The remaining eight strains, four human and four animal isolates, were clonally heterogeneous. The main group of 55 clonally related strains included isolates from human and animal infections. This result indicates that animals are a possible source of serogroup O78 septicemic *E. coli* infections in humans.

Escherichia coli strains of serogroup O78 are responsible for septicemia in calves, piglets, and lambs and for septic pericarditis and air succulatis in poultry (29). They have also been isolated from extraintestinal infections in humans (25). These pathogenic *E. coli* O78 isolates are often associated with plasmid-encoded virulence genes. In pioneer studies, W. Smith (35) showed that among 31 septicemic bovine O78 *E. coli* isolates, 25 and 4 carried ColV and Vir plasmids, respectively. More recently, Milch et al. (25) identified 13 ColV producers among 19 *E. coli* O78 isolates from human intestinal infections. Surface antigen 31A, now referred to as CS31A antigen (5, 12), has been identified in French bovine clinical isolates of serogroup O78 and in the bovine reference strain RVC 330 (19). Enterotoxigenic *E. coli* (ETEC) O78 strains with plasmid-encoded colonization factor antigen I and enterotoxins are also observed in human diarrhea (29). Such ETEC strains are usually found in developing countries and in cases of traveler's diarrhea (30).

The clonal concept allows epidemiologically unrelated clinical isolates to be grouped into clones or subclones. This concept was first suggested in 1976 by Ørskov and Ørskov (30) and then confirmed in 1983 (28). In a previous study with a small number of strains isolated in France and in Great Britain, we showed that septicemic *E. coli* O78 isolates from calves, lambs, piglets, and chickens were closely related clonally (7). The present study extends the investigation to a larger number of strains isolated in Europe and North America, including human clinical isolates belonging to septicemic or enterotoxigenic pathotypes.

MATERIALS AND METHODS

Bacterial isolates. The characteristics and origins of the 63 *E. coli* strains used are listed in Table 1. Human isolates R981, R769, and R1528 were supplied by H. Milch (National Institute of Hygiene, Budapest, Hungary); other human isolates were from the collection of I. and F. Ørskov. Porcine isolates P84-3954c, P84-5068, P86-2254, and P86-2255 and bovine isolates B79-2031, B79-2443, B78-4195, and B85-4016 were received from J. Fairbrother (Faculté de Médecine Vétérinaire, St. Hyacinthe, Québec, Canada). Porcine strains P418 and P420, bovine strains B180, B183, B185, B188, and B199, and avian strains F1, F2, F3, F30, F46, F92, and F94 were provided by M. Huggins (Houghton Poultry Research Station, Huntingdon, United Kingdom). Bovine isolates X54 and X429, ovine isolates R2, R3, and R4, and porcine isolate M10 were received from C. Wray, and bovine strains R15 and RVC 330 were from W. Sojka (Central Veterinary Laboratory, New Haw, Weybridge, United Kingdom). Bovine strains JL1, JL7, JL9, and JL10 were sent by C. Gyles (Guelph University, Ontario Veterinary College, Ontario, Canada). Other strains were isolated in France either from chickens (strains MT99, S61, and MLM) by M. Dho (INRA, Centre de Recherches de Tours Nouzilly, Monnaie, France) or from calves (strains V2019 and G2820) by L. Renault (Ets Sanders, Athis-Mons, France) or by M. Contrepois.

Most *E. coli* strains of animal origin were isolated from septicemic animals. The human *E. coli* O78 strains were isolated in Hungary or Denmark. Hungarian strains R981, R789, and R1528 were isolated during hospital outbreaks in 1974 or 1975 in Budapest from the navel or cerobrospinal fluid of infants with sepsis or from the feces of infants with enteritis. The 11 Danish strains were isolated from cases of human sepsis (strains sepsis 618, C244-67, C41-67, C248-67, and sepsis 257) or human diarrhea (strains C111-75A, C909-75, C920-75, C671-75, C790-75, and C778-74). The six latter strains are ETEC, which is considered to be noninvasive.

Serology. Serotyping was carried out by standard techniques (29). For the detection of O serogroups, bacterial cells were heated to 100°C for 1 h and then tested for agglutination with O antisera. For the detection of H serogroups, well-flagellated bacteria were selected by passing the organisms through semisolid agar before agglutination tests with H antisera.

Antimicrobial susceptibility. Antimicrobial susceptibility was tested with disks (Institut Pasteur Production, Marne la Coquette, France) by using a standard procedure (27) with Mueller-Hinton agar plates (beef infusion, 300 g liter^{-1}; Casamino Acids, 17.5 g liter^{-1}; starch, 1.5 g liter^{-1}; agar, 17 g liter^{-1} [pH 7.4]).

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TABLE 1. Phenotype characteristics of the *E. coli* O78 strains examined

Group	Isolate no. (name)	Isolate origin (country)[a]	H serogroup	Biotype[b]	OMP[c]	Esterase ET[d]	Colicin[e]	Aerobactin	Antibiotic resistance[f]
1a	1 (R981)	Human (H)	H$^-$	1	A1	1	ColV$^+$	+	A, C, T
1a	2 (R769)	Human (H)	H$^-$	1	A1	1	ColV$^+$	+	A, S, C, T
1a	3 (R1528)	Human (H)	H$^-$	1	A1	1	ColV$^+$	+	A, C, T
1a	4 (3)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	S, T, Su
1a	5 (4)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	S, T, Su
1a	6 (31)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	T, Su
1a	7 (34)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	A, S, K, C, T, Su
1a	8 (914)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	S, T, Su
1a	9 (51 arth)	Bovine (F)	H9	1	A1	1	ColV$^+$	+	S, T, Su
1a	10 (867f)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	A, S, K, C, T, Su
1a	11 (859 rate)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	A, S, K, C, T, Su
1a	12 (V2019)	Bovine (F)	H$^-$	1	A1	1	ColV$^+$	+	Su
1a	13 (R15)	Bovine (GB)	H9	1	A1	1	ColV$^+$	+	A, S, C, Su
1a	14 (B180)	Bovine (GB)	H$^-$	1	A1	1	ColV$^+$	+	A, S, C, T, Su
1a	15 (B183)	Bovine (GB)	H$^-$	1	A1	1	ColV$^+$	+	S, T, Su
1a	16 (X54)	Bovine (GB)	H$^-$	1	A1	1	ColV$^+$	+	Su
1a	17 (X429)	Bovine (GB)	H$^-$	1	A1	1	ColV$^+$	+	S, Su
1a	18 (B79-2031)	Bovine (C)	H9	1	A1	1	ColV$^+$	+	T, Su
1a	19 (B78-4195)	Bovine (C)	H9	1	A1	1	ColV$^+$	+	S, Su
1a	20 (R2)	Ovine (GB)	H$^-$	1	A1	1	ColV$^+$	+	S, Su
1a	21 (R3)	Ovine (GB)	H$^-$	1	A1	1	ColV$^+$	+	S, C, T, Su
1a	22 (R4)	Ovine (GB)	H$^-$	1	A1	1	ColV$^+$	+	S, Su
1a	23 (MT99)	Avian (F)	H$^-$	1	A1	1	ColV$^+$	+	S, T, Su
1a	24 (MLM)	Avian (F)	H$^-$	1	A1	1	ColV$^+$	+	
1a	25 (F2)	Avian (GB)	H$^-$	1	A1	1	ColV$^+$	+	T, Su
1a	26 (S41)	Avian (GB)	H$^-$	1	A1	1	ColV$^+$	+	
1a	27 (P418)	Porcine (GB)	H$^-$	1	A1	1	ColV$^+$	+	A, S, K, Su
1a	28 (sepsis 618)	Human (D)	H$^-$	1	A1	2	ColV$^+$	+	T, Su
1a	29 (C244-67)	Human (D)	H$^-$	1	A1	2	ColV$^+$	+	A, S, K, C
1a	30 (C41-67)	Human (D)	H$^-$	1	A1	2	ColV$^+$	+	A
1a	31 (C248-67)	Human (D)	H$^-$	1	A1	2	ColV$^+$	+	A
1a	32 (M10)	Porcine (GB)	H$^-$	1	A1	2	ColV$^+$	+	S, T, Su
1b	33 (B85-4016)	Bovine (C)	H$^-$	1	A2	1	ColV$^+$	+	T, Su
1b	34 (B79-2443)	Bovine (C)	H$^-$	1	A2	1	ColV$^+$	+	A, S, C, Su
1b	35 (F94)	Avian (GB)	H9	1	A2	1	ColV$^+$	+	S, T, Su
1b	36 (F46)	Avian (GB)	H$^-$	1	A2	2	ColV$^+$	+	T, Su
1b	37 (F92)	Avian (GB)	H$^-$	1	A2	2	ColV$^+$	+	T
1b	38 (P84-5068)	Porcine (C)	H$^-$	1	A2	1	ColV$^+$	+	T, Su
1b	39 (P84-3954C)	Porcine (C)	H$^-$	1	A2	1	ColV$^+$	+	S, K, C, T, Su
1c	40 (F1)	Avian (GB)	H9	1	A3	1	ColV$^+$	+	S, T, Su
1c	41 (F3)	Avian (GB)	H9	1	A3	1	ColV$^+$	+	T, Su
1d	42 (P420)	Porcine (GB)	H9	1	A4	1	Col$^+$	+	A
1d	43 (P86-2255)	Porcine (C)	H$^-$	1	A4	1	Col$^-$	−	S, K, T, Su
1d	44 (P86-2254)	Porcine (C)	H$^-$	1	A4	1	Col$^-$	+	S, K, T, Su
1d	45 (S61)	Avian (F)	H9	1	A4	1	Col$^+$	−	T
1d	46 (G2820)	Bovine (F)	H$^-$	1	A4	2	Col$^-$	+	A
1d	47 (JL9)	Bovine (GB)	H$^-$	1	A4	2	Col$^-$	+	
1d	48 (1404)	Bovine (F)	H$^-$	1	A4	2	Col$^-$	−	
1d	49 (B199)	Bovine (GB)	H$^-$	1	A4	2	Col$^+$	−	
1d	50 (B185)	Bovine (GB)	H$^-$	1	A4	5	Col$^-$	+	Su
1d	51 (RVC 330)	Bovine (GB)	H$^-$	1	A4	4	Col$^-$	−	
1e	52 (C111-75A)	Human (D)	H12	1	A5	3	Col$^-$	−	
1e	53 (C909-75)	Human (D)	H$^-$	1	A5	3	Col$^-$	−	
1e	54 (C920-75)	Human (D)	H$^-$	1	A5	3	Col$^-$	−	
1e	55 (B188)	Bovine (GB)	H$^-$	1	A5	1	Col$^-$	−	T
2	56 (JL10)	Bovine (GB)	H$^-$	2	B	4	Col$^-$	−	
3	57 (JL1)	Bovine (GB)	H2	2	C	6	Col$^+$	+	S, T, Su
3	58 (JL7)	Bovine (GB)	H2	2	C	7	Col$^+$	+	S, T, Su
3	59 (F30)	Avian (GB)	H4	2	C	7	Col$^+$	+	S, T, Su
4	60 (C671-75)	Human (D)	H12	3	D	8	Col$^-$	−	
5	61 (C790-75)	Human (D)	H11	4	E	8	Col$^-$	−	
5	62 (C778-74)	Human (D)	H11	4	E	8	Col$^-$	−	
6	63 (sepsis 257)	Human (D)	H$^-$	5	F	9	Col$^+$	−	C

[a] Countries: H, Hungary; F, France; GB, Great Britain; D, Denmark; C, Canada.
[b] See Table 2 for biotypes.
[c] See Fig. 1 for OMP patterns.
[d] See Table 3 for esterase ETs.
[e] Col$^+$ indicates colicin produced differently than colicin V; Col$^-$, no colicin produced.
[f] Abbreviations: A, ampicillin; S, streptomycin; K, kanaymcin; C, chloramphenicol; T, tetracycline; Su, sulfamide.

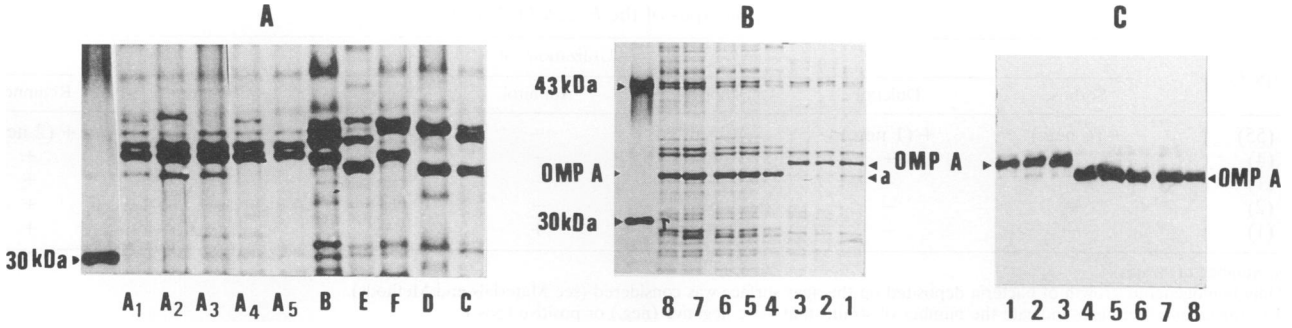

FIG. 1. (A) Representative patterns of the major OMPs of the *E. coli* serogroup O78 strains; (B and C) identification of OMPA and the plasmid-encoded OMP. (A) Different OMP patterns identified in this study. (B) Silver-stained OMP patterns of strains F94 (lane 1), F1 (lane 2), R2 (lane 3), and their ColV$^+$ transconjugants K-12(pColV F94) (lane 4), K-12(pColV F1) (lane 5), and K-12(pColV R2) (lane 6); OMPs of strains K-12 and ColV reference strain K-12(pColV K30) are shown in lanes 8 and 7, respectively. (C) Western blot (immunoblot) identifying OMPA in the patterns shown in panel B (lanes 1 to 8). Plasmid-encoded protein is visible in wild-type strains (a) but is hidden by OMPA in K-12 ColV$^+$ transconjugants.

Utilization of sugar. Growth on agar minimal medium (KH_2PO_4, 1.36 g liter^{-1}; $Na_2HPO_4 \cdot 2H_2O$, 10.1 g liter^{-1}; $(NH_4)_2SO_4$, 2 g liter^{-1}; $MgSO_4 \cdot 7H_2O$, 10^{-2} g liter^{-1}; $MnCl_2 \cdot 4H_2O$, 10^{-3} g liter^{-1}; $FeCl_3 \cdot 6H_2O$, 1.35 $\times$ 10^{-4} g liter^{-1}; $CaCl_2 \cdot 2H_2O$, 4 $\times$ 10^{-4} g liter^{-1}) supplemented with 5 g of either sorbose, dulcitol, inositol, adonitol, sucrose, raffinose, or rhamnose per liter of filtered solution was observed after 48 h of incubation at 37°C. The composition is derived from the mineral base for Minca medium (17). A control with glucose identified isolates with growth factor requirements. Bacteria were spotted on the surface of the agar. After 48 h, we observed either homogeneous bacterial growth or a few isolated colonies, which suggested that the bacterial population was heterogeneous.

Colicin production. Colicin production was measured by a double-layer method (11). The spot-cultured *E. coli* isolates grown on the surface of a Luria-Bertani agar medium were lysed by $CHCl_3$ vapor. The production of colicin was evidenced in a second layer of semisolid agar seeded with *E. coli* K-12 DB6433 (8) sensitive to most colicins. The inhibition of *E. coli* K-12 growth indicated the production of a colicin. Colicin V was identified by the same technique but by the use of a mutant of the *E. coli* K-12 strain that was resistant to colicin V (ColVr) or of the *E. coli* K-12 strain carrying a ColV plasmid. The colicin-producing strains which did not inhibit *E. coli* K-12 ColVr and the immune *E. coli* K-12(pColV) were considered to be colicin V producers.

Aerobactin production. Aerobactin production was determined by the Csaky assay (6), which identifies hydroxamate compounds. In addition, aerobactin production was detected by the cross-feeding test described by Carbonetti and Williams (3). The indicator strain *E. coli* LG1522 is a mutant impaired in the uptake of enterochelin and the synthesis of aerobactin, but the receptor of the ferric-aerobactin complex is not affected.

Crude outer membrane protein (OMP) analysis. Bacteria grown on the surface of Minca agar medium (17) for 18 h at 37°C were collected in 2 ml of sterile saline and centrifuged for 15 min at 4,500 $\times$ *g*. The pellet was then suspended in 10 ml of Tris buffer at 4°C (0.05 M Tris base, 1 mM EDTA [pH 7.8] with HCl). Sonication with a 15% continuous cycle was performed on ice for four periods of 15 s (New Brunswick). After centrifugation for 20 min at 1,200 $\times$ *g*, the supernatant was centrifuged at 50,000 $\times$ *g* for 1 h at 4°C. The pellet was suspended in 10 mM Tris buffer with 5 mM $MgCl_2$ (pH 8) containing 2% Triton X-100 to render the inner membrane soluble (33). After centrifugation at 50,000 $\times$ *g* for 1 h at 4°C, the pellet was dissolved in buffer (0.025 M Tris base, 0.192 M glycine, 1% sodium dodecyl sulfate (SDS) (pH 8.6). Polyacrylamide gel electrophoresis (PAGE) was performed after the addition of Laemmli buffer (23) and heating at 100°C for 5 min. A 10% polyacrylamide gel was used, and electrophoresis was run at 20 mA for 5 h. Silver staining of the gel was done as described by Oakley et al. (26). Immunoblotting analysis of OMP type A (OMPA) was made after electrophoretic transfer to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.) as described by Towbin et al. (36). The polyclonal rabbit anti-OMPA antiserum (a gift from J. P. Girardeau) was diluted 1/500. Peroxidase-conjugated goat anti-rabbit immunoglobulin G (Nordic Immunological Laboratories, Tillburg, The Netherlands) and 4-chloro-1-naphthol (Bio-Rad) were used to detect rabbit antibodies.

Electrophoretic analysis of esterases. *E. coli* strains were cultured in Fernbach flasks containing 500 ml of L broth (24) without glucose. The flasks were shaken vigorously for 18 h at 37°C in a reciprocating water bath shaker set at about 70 oscillations min^{-1}. The preparation of extracts, protein estimation, horizontal slab PAGE (7% [wt/vol] acrylamide, Tris-glycine buffer [pH 8.6]), the estimation of electrophoretic mobility, and esterase staining have been described previously (13). Each electrophoretic mobility variant was designated as an allozyme. When an esterase was not detected in a strain, it was recorded as a null allozyme. Each distinctive combination of allozymes for the four varieties of esterases (B, A, C, and I) was designated an electrophoretic type (ET) (14).

Statistical analysis. The data were summarized in a two-way table of 63 rows, one for each strain, and 32 columns corresponding to the number of H serogroups, biotypes, OMP patterns, esterase ETs, and the presence of ColV and aerobactin. From this table, a dissimilarity matrix was constructed for hierarchical clustering with a model MP100-60 Olivetti computer by using STAT-ITCF software (18, 20).

RESULTS

The origins and characteristics of the 63 *E. coli* isolates are summarized in Table 1. The 63 isolates were categorized into six groups, 1 to 6, on the basis of OMP patterns A, B, C, D, E, and F, respectively (Table 1; Fig. 1A). Group 1, the largest, comprising 55 strains isolated in Europe or North America from human, bovine, ovine, porcine, and avian species, had a type A OMP pattern, H$^-$ or H9 flagellar antigen (except H12

TABLE 2. Biotypes of the *E. coli* O78 strains

Biotype (*n*)[a]	Utilization[b] of:						
	Sorbose	Dulcitol	Inositol	Adonitol	Sucrose	Raffinose	Rhamnose
1 (55)	+ (4 neg.)[c]	+ (1 neg.)	Variable	−	Variable	+ (3 neg.)	+ (2 neg.)
2 (4)	−	+	− (1 pos.)	−	+	+	+
3 (1)	−	−	−	−	+	+	+
4 (2)	−	−	−	−	−	−	+
5 (1)	−	−	−	+	−	−	+

[a] *n*, number of strains.
[b] Only homogeneous growth of bacteria deposited on the agar surface was considered (see Materials and Methods).
[c] The values in parentheses indicate the number of strains that were negative (neg.) or positive (pos.).

of strain C111-75A), and a type 1 biotype. Group 1 was subdivided into five subgroups, 1a, 1b, 1c, 1d, and 1e, to take into account minor differences between the OMP patterns, i.e., A1, A2, A3, A4, and A5, respectively (Fig. 1A). Biotypes (Table 2) and ETs of esterases (Table 3) were also studied. In the OMP pattern type A, three main bands with the same median molecular weights were present in subgroups A1, A2, A3, A4, and A5, which were differentiated by the presence or absence of high- or low-molecular-weight OMPs or both. ColV$^+$ strains of subgroups 1a, 1b, and 1c had a low-molecular-weight OMP, but ColV$^-$ strains of subgroups 1d and 1e did not. The plasmid-encoded aerobactin production demonstrated by Williams (41) also strongly correlated with the ColV$^+$ status of the strains. We tried to demonstrate that the lowest-molecular-weight OMP of ColV$^+$ strains is plasmid encoded (Fig. 1B and C) by a comparison of OMPs in *E. coli* K-12 and transconjugant K-12(pColV), but the plasmid-encoded OMP was hidden by OMPA of *E. coli* K-12. Such a plasmid-encoded OMP has been identified previously and called VmpA by Rowburry et al. (32) or PCP by Achtman et al. (1). The electrophoretic distribution of esterase B, A, C, and I allozymes was also homogeneous for the strains of group 1. Among the ColV$^+$ strains of subgroups 1a, 1b, and 1c, ETs 1 and 2, differing only in esterase I, were encountered. Among the *E. coli* ColV$^-$ strains of subgroups 1d and 1e, ETs 1, 2, 4, and 5 and ETs 1 and 3 were found, respectively. Three strains of subgroup 1e were human ETEC. All of the strains of subgroups 1a, 1b, 1c, and 1d were isolated either from extraintestinal sites or from the intestine. We considered the strains from the intestine to be septicemic *E. coli*. These isolates were not screened for enterotoxic activity, but investigations for fimbrial antigens did not identify K99, K88, F41, or 987P, which are characteristic attributes of animal ETEC. Fimbrial or protein-capsular antigens were identified only in strain 1404 (Vir antigen) and in strains G2820 and RVC 330 (CS31A antigen). Most of the other strains of subgroups 1a to 1d produced fimbriae with subunits having apparent molecular sizes in the range of 20 kDa. The results of the fimbrial study are not shown since they are similar to those previously published by Dassouli-Mrani-Belkebir et al. (7) and Contrepois et al. (5). None of the strains produced K antigen.

The eight other ColV$^-$ strains were assigned to groups 2, 3, 4, 5, and 6. They were heterogeneous for OMP patterns as well as for H serotypes, biotypes, and esterase ETs. Strains C671-75 (group 4), C790-75, and C778-74 (group 5) were human ETEC. A few of the ColV$^-$ strains produced colicin or aerobactin or both, but none of these proteins was produced by human ETEC.

The dissimilarity between strains was used to construct a dendrogram (Fig. 2) which essentially corresponds to the proposed classification. The sole discrepancy concerns the strains B185 and RVC 330, which were differentiated from the other strains by their OMP pattern A4 and by their esterase ETs, i.e., 5 and 4, respectively.

TABLE 3. ETs of esterases B, A, C, and I

ET	Electrophoretic mobility of esterase			
	B	A	C	I
1	70	0	57	60
2	70	0	57	68
3	70	81	57	60
4	70	81	57	68
5	72	0	57	68
6	72	81	57	0
7	70	81	0	70
8	68	75	48	70
9	70	75	48	65

DISCUSSION

Serotypes, biotypes, and OMP patterns are used to describe *E. coli* clonal groups (2). The application of multilocus enzyme electrophoresis for identifying and epidemiologically tracing

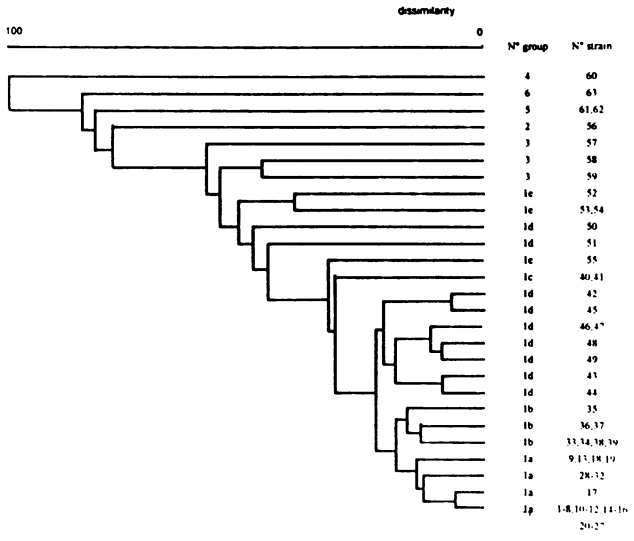

FIG. 2. Dendrogram prepared from the distance obtained from the dissimilarity matrix on the basis of the phenotypic characteristics of the *E. coli* O78 strains examined. The group numbers and the strain numbers (shown in Table 1) are indicated for each cluster.

pathogens has established that, for many bacterial species, a few geographically widespread clones account for most cases of infectious disease (2, 34).

E. coli strains of serogroup O78 were a good candidate for a clonal study because a previous investigation involving a small number of strains showed that *E. coli* O78 strains isolated from animals in western Europe were clonally related (7). The results of this preliminary study also suggested that *E. coli* O78 clones could be classified by virulence plasmids encoding either ColV or Vir phenotypes (35), a CS31A phenotype (12), or colonization factor antigen and enterotoxigenicity (10). This hypothesis could not be definitely confirmed in the present study because only two strains producing CS31A (G2820 and RVC 330) and one strain producing the Vir fimbriae (strain 1404) were identified. These three strains were not notably different from O78 ColV$^+$ strains, which are homogeneous and clonally related. The results for human ETEC indicated that strains C111-75A, C909-75, and C920-75 clustered in subgroup 1e, which is related to the other subgroups 1a to 1d. These three serogroup O78 human ETEC strains are thus not clearly distinct clonally from septicemic serogroup O78 *E. coli* strains. In contrast, three other human serogroup O78 ETEC isolates (C671-75, C790-75, and C778-74) were assigned to specific clones 4 and 5, which are unrelated to other clones and subclones delineated in the present work.

We distinguished five subgroups or subclones 1a to 1e within the 55 strains of group 1, mainly on the basis of the OMP patterns. The OMPs of subgroups 1d and 1e differed from those of subgroups 1a and 1c, respectively, only in the absence of the lowest-molecular-weight OMP, which is plasmid encoded. Only genomic traits are considered for clonal grouping. Consequently, subgroups 1a and 1d are very similar, as are subgroups 1c and 1e. This conclusion is also reinforced by statistical analysis. In the dendrogram in Fig. 2, the strains of subgroup 1d are clustered near those of subgroups 1a and 1b, except for isolates 50 and 51, which have different ETs. However, the clustering of subgroups 1c and 1e, which are separated by isolates 50 and 51 and those of group 3, is more difficult to explain.

It has been shown that esterase polymorphism divides *E. coli* strains into two groups, B_1 and B_2, by the pattern of mobility of esterase B (15, 16). The highly pathogenic B_2 strains exhibit several chromosomally encoded virulence factors, whereas certain B_1 strains possess some plasmid-encoded virulence factors (21). The present results are in agreement with these findings since most of the *E. coli* O78 strains which exhibited a B_1 pattern were associated with virulence plasmids pColV, pCS31A, or pVir.

Most *E. coli* serotype isolates from septicemia belong to O groups, which ordinarily have acidic polysaccharide capsules (31). It is noteworthy that the invasive *E. coli* O78 isolates have no special K polysaccharide.

Interestingly, *E. coli* O78 strains isolated from poultry have been studied clonally by different authors, and comparisons can be made. On the basis of multilocus enzyme electrophoresis, clonal relationships among *E. coli* isolates from turkey septicemia and avian diseases were recently studied by White et al. (37–39). These studies assigned all of the *E. coli* O78 isolates from chickens or turkeys, including strain MT99 in the article by White et al. (37), to clone complex B3 grouping related subclones. We assigned strain MT99 (no. 23 in Table 1) to the large clonal group 1. This analogy between classifications suggests that clone complex B3 as defined by White et al. for *E. coli* strains isolated from poultry in France, Saudi Arabia, and the United States also includes septicemic *E. coli* O78 strains isolated from humans and other animal species in Europe and Canada.

OMP patterns of *E. coli* O78 strains that cause avian septicemia have also been studied by Kapur et al. (22) and Dho-Moulin et al. (9). It is difficult to compare the OMP patterns obtained in the former study with ours. The technical reasons for the differences between the patterns (preparation of OMP extracts and silver or Coomassie blue staining) have already been mentioned by Kapur et al. (22) in a discussion comparing their results with those of Dho-Moulin et al. (9). We used the same methods as those of Dho-Moulin et al. for the analysis of OMPs. Pattern 5, obtained by Dho-Moulin et al. (9) for *E. coli* O78 strains including isolate MT99, corresponds to OMP type A1 of strains grouped in subclone 1a in our study.

In conclusion, different clones correlating with the H serogroups were identified among 63 *E. coli* strains isolated in Europe and North America. However, a major group of related subclones delineating 55 strains with O78:H$^-$ or H9 serotypes was identified. A comparison of our results with other published works suggests that these 55 strains belong to a group of closely related subclones probably analogous to the B3 clone complex identified by Whittam et al. (40) and White et al. (39) among pathogenic *E. coli* O78 strains isolated from poultry. The worldwide clonal relatedness observed among septicemic *E. coli* O78 strains isolated from poultry (37) also holds for *E. coli* O78 strains isolated from human, bovine, ovine, and porcine septicemia. This finding indicates that the risk of zoonosis exists with septicemic *E. coli* O78 strains. Virulence studies with animal models have shown that the septicemic potency of *E. coli* O78 isolates is always high (7, 37). The zoonotic risk already identified for *E. coli* O2:K1 (1) and septicemic serogroup O6 isolates (4) is also present with septicemic *E. coli* O78 isolates.

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