

Pathogenicity characteristics of uropathogenic *Escherichia coli* strains

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ABSTRACT

Seventeen wild-type uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infection (UTI) and one pyelonephritis strain were studied for pathogenicity traits. Their plasmid, outer membrane protein, surface protein and RAPD DNA profiles, hemolysin and colicin production, type of fimbriae expressed and capacity of adhesion to uroepithelial cells were determined in an attempt to correlate these characteristics with pathogenicity. The only characteristic that had a consistent pattern in these types of *E. coli* was adhesion to uroepithelial cells.

INTRODUCTION

Escherichia coli is one of the most prevalent infectious agents for humans. It is able to cause diarrheogenic diseases, neonatal meningitis and urinary tract infections (Levine, 1987). Urinary tract infections (UTI) are the most common form of extraintestinal *E. coli* infection, and *E. coli* is the most common cause of UTI (Johnson, 1991). During their lives, at least 12% of men and 10 to 20% of women experience an acute symptomatic UTI (Johnson and Stamm, 1989; Lipsky, 1989), and an even greater number develop asymptomatic bacteriuria (ABU). In the United States, annually, more than 100,000 patients are hospitalized because of renal infection (Johnson and

Stamm, 1989) with its attendant risk of gram-negative sepsis and death (Johnson, 1991). The great public health importance of this kind of infection has led to many studies on the pathogenicity mechanisms of uropathogenic *E. coli* (Orskov and Orskov, 1985; Harber *et al.*, 1986; Eisenstein and Jones, 1988; Orskov *et al.*, 1989; Johnson, 1991). The capacity of superficial proteic appendages (fimbriae) expression is known to be a crucial step for the colonization of the uroepithelial tract mucosal surface (Reid and Sobel, 1987).

In this work we studied 17 cystitis-wild-type uropathogenic *E. coli* strains which were classified according to the clinical characteristics observed in the urine of the patients, such as BWL (bacteria without leukocytes), BL (bacteria with leukocytes) or BLR (bacteria with leukocytes and red blood cells), and one pyelonephritis-septicemic strain isolated at the Faculdade de Ciências Médicas (FCM) of the Universidade Estadual de Campinas (UNICAMP). We also examined genetic characteristics (antibiotic resistance levels, plasmid profile, colicin and hemolysin production), capacity of red blood cell (RBCs) agglutination, outer membrane protein profile, surface

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proteins which are expressed at 37°C (but not at 16°C), and RAPD DNA fingerprinting patterns in an attempt to correlate virulence factors with pathogenicity.

MATERIAL AND METHODS

Bacterial strains and media

The 17 wild-type uropathogenic *E. coli* strains were isolated from patients with urinary tract infection (UTI) at the FCM, UNICAMP, Campinas, Brazil, and were classified as BWL (strains 63, 66, 154, 177, 202), BL (strains 7, 9, 28, 46, 168, 178, 189), BLR (strains 33, 70, 81, 121, 198), and DT (septicemic-pyelonophritis strain) (Table II). Other strains used in this work are described in Table I. CFA (Evans *et al.*, 1979), LB and LA media (Sambrook *et al.*, 1989) were used for bacterial growth. All the strains were stored at -70°C in LB plus 15% glycerol to avoid plasmid loss. Antimicrobial drugs (Sigma) used in this study were ampicillin (Ap), streptomycin (Sm), chloramphenicol (Cm), tetracyclin (Tc), kanamycin (Km), and nalidixic acid (Na).

Antimicrobial drug resistance determination

The level of resistance to the different antimicrobial drugs was determined by growing each strain overnight (37°C) as a preinoculum in LB medium and

then adding 10 µl (approximately 10⁷ cells/ml) of this culture in LB medium with increasing concentrations (0, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 µg/ml) of the antimicrobial drugs and incubating these tubes at 37°C overnight. The level of resistance was considered as the highest concentration with bacterial growth. Strains HB101, C600 and MS101 were used as sensitivity controls.

Hemagglutination and expression of fimbriae

The expression of type 1 fimbriae or other fimbriae types on the surface of the different strains was verified by agglutinating human and guinea pig red blood cells either in the presence or in the absence of D-mannose, as described by Evans *et al.* (1981). Type P fimbriae were determined by using a commercial P-fimbriae specific agglutination test, as described by Blanco *et al.* (1992).

Plasmid extraction and agarose gel electrophoresis

Plasmid extraction was carried out by the small scale alkaline lysis method described by Birnboim and Doly (1979) and gel electrophoresis was conducted on 0.7% agarose gels as described by Sambrook *et al.* (1989). V517 strain (Table I) was used as a carrier strain for plasmids of known size.

Table I - Strains used as controls in this work.

HI10407	CFA/1, LT ⁺ , ST ⁺ (Evans <i>et al.</i> , 1975, 1978)
O157:H7	Enterohemorrhagic <i>E. coli</i> strain isolated from an outbreak of hemorrhagic diarrhea in Brazil
O55:H6	Enteropathogenic <i>E. coli</i> strain with localized adherence on HeLa cells isolated from an outbreak of infantile diarrhea in Brazil
ORN115	Type 1 fimbriae (Orndorff <i>et al.</i> , 1985)
C600	Cm ^r , supE44, hsdR, thi-1, thr-1, leuB6, lacY1, tonA21 (Young and Davis, 1983)
HB101	Sm ^r , supE44, dsdS20 (r ⁻ , m ⁻), recA13, ara-14, proA2, lacY1, galK2, rpsL20, xyl-5, mtl-1 (Boyer and Roulland-Dussoix, 1969; Bolivar and Backman, 1979)
DH5α	F ⁻ , endA1, hsdR17 (rk ⁻ , mk ⁺) supE44, thi-1, girA96, relA1, (Hanahan, 1983)
V517	Strain harboring plasmids with known molecular weight (32MD, 5.19MD, 3.48MD, 3.03MD, 2.24MD, 1.69MD, 1.51MD, 1.25MD) was a gift from Dr. James B. Kaper, Center for Vaccine Development, USA
MS101	<i>E. coli lac⁻</i> (Dept. de Microbiologia e Imunologia - UNICAMP)

Table II - Characteristics of strains studied in this work.

Strain	Groups	AR	CP	UA	H	P	I	X/Dr
7	BL	Tc, Sm, Ap, Na, Cm	-	36	+	ND	-	-
9	BL	Sensitive	-	18	-	-	-	-
28	BL	Tc, Sm, Ap, Na, Cm	-	13	+	+	+	?
46	BL	Sm, Ap, Cm	-	62	+	+	+	?
168	BL	Tc, Sm, Ap, Km	-	45	+	-	+	-
178	BL	Tc, Sm, Ap, Cm	-	18	-	+	+	?
189	BL	Tc, Sm, Ap, Na, Cm	+	17	+	ND	+	+
63	BWL	Na	+	19	-	+	-	?
66	BWL	Sm, Na	+	25	+	-	+	+
154	BWL	Na	-	8	+	-	+	-
177	BWL	Tc, Sm, Na	+	24	-	-	-	-
202	BWL	Sm, Ap, Na	+	15	-	-	+	+
33	BLR	Sm, Ap, Na	+	16	-	+	-	?
70	BLR	Tc, Na	-	20	+	ND	-	?
81	BLR	Sensitive	+	20	-	+	-	?
121	BLR	Sm, Ap	+	26	+	+	+	?
198	BLR	Tc, Sm, Ap, Cm	-	8	-	-	+	-
DT		Tc, Sm, Ap, Na	-	63	+	-	-	ND
ORN115			-	7	-	-	+	-

BL - Bacteria with leukocytes, **BWL** - bacteria without leukocytes, **BLR** - bacteria with leukocytes and red-blood cells, **Ar** - antibiotic resistance, **Ap** - ampicillin, **Sm** - streptomycin, **Tc** - tetracyclin, **Cm** - chloramphenicol, **Km** - kanamycin, **Na** - nalidixic acid, **CP** - colicin production, **UA** - uroepithelial adhesion (mean observed in 40 cells), **H** - hemolysin production, **I** - type 1 fimbriae, **P** - type P fimbriae, **X/Dr** - Dr or X adhesins, **ND** - not determined, ? - May or not have mannose-resistant hemagglutination (MRHA) adhesins other than P fimbriae.

Superficial protein extraction

Superficial protein extraction was carried out as described by Sperandio and Silveira (1993) from strains grown on CFA agar at 16°C and at 37°C.

Outer membrane protein extraction

Outer membrane protein extraction was carried out as described by Silveira *et al.* (1994).

SDS-PAGE electrophoresis

Superficial protein and outer membrane protein electrophoreses were carried out using Laemmli's system (1970), in a Pharmacia 2001 System electrophoresis apparatus. The gel preparation and staining followed the methodology described in the Hoefer Scientific Instruments (1990), and Blum *et al.* (1987), respectively.

Colicin production determination

Colicin production was determined by the method described by Azevedo and da Costa (1973).

Hemolysin production

Hemolysin production was assayed by growing the different strains in LB medium overnight (37°C) and dropping 50 µl of this growth on a Petri dish containing sheep-blood agar. This culture was incubated at 37°C overnight and the hemolysin production verified by the presence of a hemolysis halo.

Adherence assay

The adhesion capacity of the different bacterial strains to uroepithelial cells was assayed as described by Svanborg-Eden (1986) and Svanborg-Eden *et al.* (1988). Briefly, squamous and transitional epithelial

cells from the sediment of the urine of one female donor were suspended in PBS. Bacteria (10^8) were added to 10^5 epithelial cells in PBS with D-mannose diluted to a volume of 1 ml. After rotation for 60 min at 37°C unattached bacteria were eliminated by repeated washing and the cells fixed and gram stained. The number of bacteria attached to the epithelial cells was counted by directed light microscopy. Adhesion was given as the mean number of bacteria attached to 40 epithelial cells. Strain ORN115 was used as the type 1 fimbriae standard.

RAPD fingerprinting (PCR)

PCR was carried out as described by Williams *et al.* (1990) in a final volume of 100 μl , containing 100 ng of primer (5'TGCCGAGCTG3'), 1U of TaqDNA polymerase (Gibco/BRL) with the reaction conditions described by the manufacturer, 500 μM each of dCTP, dGTP, dATP and dTTP (Pharmacia) and 5 μl of boiled bacterial cells. Briefly, boiled bacterial cells were prepared as follows: 100 μl of an overnight culture (37°C) was added to a Petri dish containing LA medium and this was kept at 37°C overnight. A suspension containing approximately 10^{10} cells/ml was made in TE (10 mM Tris HCl, pH 8.0; 1 mM EDTA) buffer and 5 μl was boiled for 10 min with all the amplification reagents, except the enzyme. The reaction mixture was kept at room temperature for 10 min, spun quickly, and 1 U of the enzyme and a drop of mineral oil added. A thermal cycler was used for amplification. The cycling program was one cycle (94°C , 3 min) and 45 cycles (94°C , 1 min; 34°C , 1 min, and 72°C , 2 min). After PCR, 10 μl of the products was electrophoresed in 1% agarose gels containing 0.5 $\mu\text{g}/\text{ml}$ ethidium-bromide, and the result photographed under UV light. Strains HB101, DH5 α , H10407, 0157:H7 and 055:H6 were used as a control.

RESULTS

Table II shows the data obtained with the assays for antimicrobial drug resistance, hemolysin and colicin production, adherence to uroepithelial cells, and hemagglutination using guinea pig and human red blood cells (RBCs) with and without D-mannose (type 1 fimbriae) and type P-fimbriae. Most of the strains were resistant to at least one drug, with the exception of strains 9 (BL) and 81 (BLR). Strains from group BL had the most extensive drug resistances.

Among 18 strains, eight were able to produce colicin, colicins E1, E3 and V being the most prevalent

ones (results not shown). In the BL group only one strain (189) was a colicin producer.

The hemolysin production assay demonstrated that 10 strains were hemolysin producers, and group BL had the largest number of strains expressing hemolysin.

The hemagglutination assays showed that 10 strains were able to express type 1 fimbriae and that they (strains 28, 46, 66, 121, 178, 189, 202) or not (strains 154, 168, 198) could produce another type of hemagglutination factor. On the other hand, there were strains that could not produce type 1 fimbriae but could (strains 33, 63, 70, 81) or not (9, 177, DT) express another type of hemagglutination factor.

Extraction and gel electrophoresis of plasmid DNA (Figures 1 and 2) showed that all strains, except strain 70, had plasmids of different molecular weights. Strains 28 (BL) and 46 (BL) had an identical plasmid DNA profile (Figure 2) and strains 202 (BWL) and 33 (BLR) had a very similar DNA profile (Figure 1).

SDS-PAGE gel electrophoresis of outer membrane proteins gave exactly the same profile for all the strains (data not shown).

Comparison of SDS-PAGE gel electrophoresis of expressed proteins from the surface of the bacteria grown either at 37°C or 16°C allowed us to classify these strains into eight groups (Figure 3).

The adherence to uroepithelial cells assay showed that all the strains had the capacity of adhesion (Table II) to this kind of cell. Strains 46 and DT had the highest adhesion capacity and strains ORN115, 198, 154 (type 1 producers) had the lowest.

The RAPD fingerprinting assay demonstrated that most strains had a profile similar to strain 63 (28,

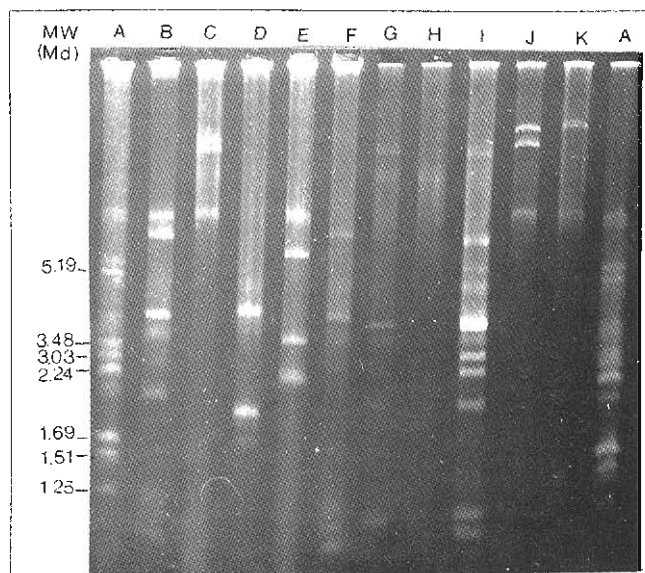


Figure 1 - Agarose (0.7%) gel electrophoresis of plasmid DNA of strains: A(V517), B(63), C(66), D(177), E(178), F(202), G(33), H(70), I(81), J(121), K(198).

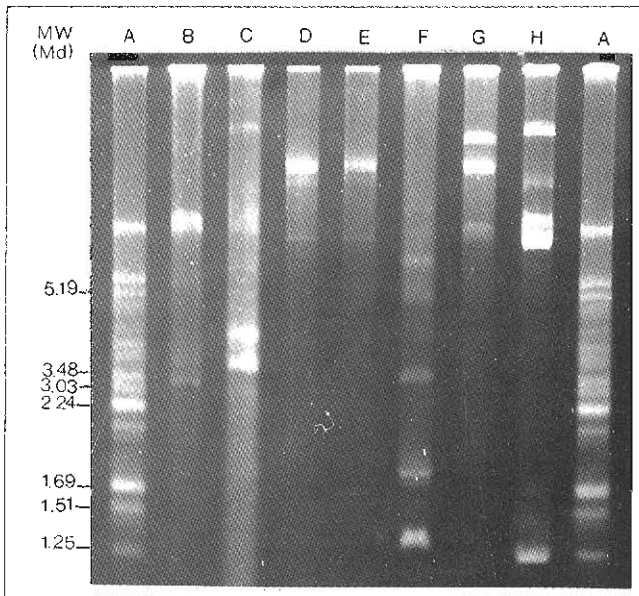


Figure 2 - Agarose (0.7%) gel electrophoresis of plasmid DNA of strains: A(V517), B(7), C(9), D(28), E(46), F(168), G(189), H(DT).

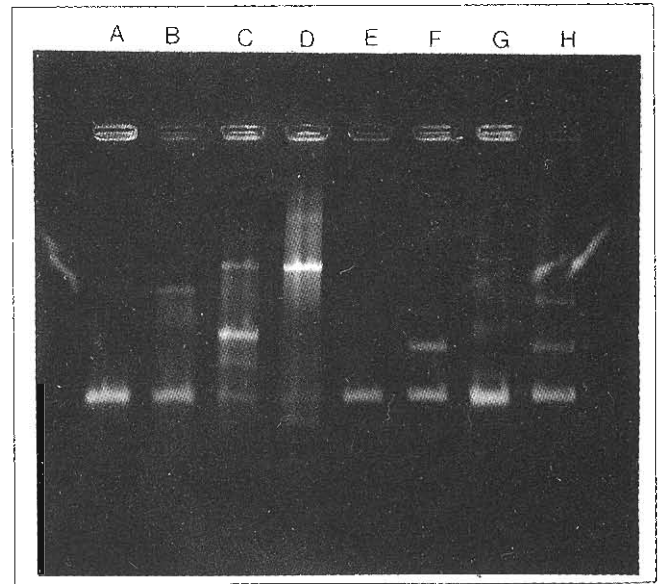


Figure 4 - Agarose (1%) gel electrophoresis of amplified DNA using the RAPD DNA fingerprinting technique. A(H10407), B(DH5α), C(055:H6), D(0157:H7), E(63), F(178), G(198), H(DT).

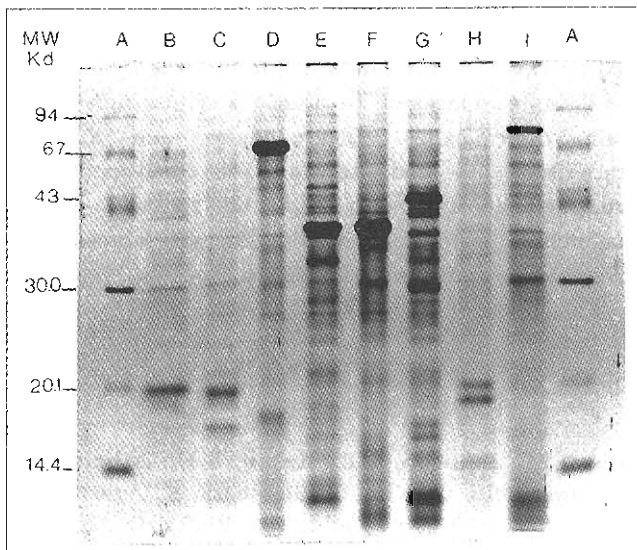


Figure 3 - SDS-PAGE (13%) electrophoresis of surface proteins expressed at 37°C (but not at 16°C) of strains: A (molecular weight markers), B(46), C(70), D(168), E(66), F(198), G(121), H(DT), I(202).

33, 46, 70, 81, 121, 154, 168, 177, 189, 202, H10407, HB101) while strains DH5α, 055:H6, 0157:H7, 178 (66), DT (7) and 198 were different (Figure 4).

DISCUSSION

Several virulence factors are possibly associated with the pathogenicity of *Escherichia coli* strains that cause urinary tract infections (UTI). These

factors include hemolysin production (Hughes *et al.*, 1983; Arthur *et al.*, 1989) which could be associated with other virulence factors, such as fimbriae (Low *et al.*, 1984; High *et al.*, 1988), epithelial cell adherence (Svanborg Eden *et al.*, 1988), serum resistance (Hughes *et al.*, 1982), certain O groups (Evans *et al.*, 1981; Hughes *et al.*, 1982; Czirik *et al.*, 1986), and certain K antigens (Evans *et al.*, 1981; Hacker *et al.*, 1986). Hemolytic strains are normally more virulent than nonhemolytic ones (Hacker *et al.*, 1986), because hemolysin liberates the host's iron by disruption of erythrocytes and other cells (MacKenzie and O'Hanley, 1991).

Vaisanen-Rhen *et al.* (1984) and Cavalieri and Snyder (1982) correlated the production of hemolysin with pathogenicity of uropathogenic *E. coli* strains. Among our uropathogenic isolates we found strains that were able (10) or not (eight) to produce hemolysin. We believe that, at least for this characteristic, hemolysin is not essential for pathogenicity. Hemolysin production was most prevalent in the BL group, but not a characteristic trait for this group, since the other two groups also had strains that were able to express hemolysin production.

All strains (but strain 70) had at least one plasmid and the presence of this extrachromosomal DNA could be responsible for the multiple drug resistance found in almost all strains. The existence of an identical plasmid DNA profile and an almost identical drug resistance pattern for strains 28 and 46

(BL group) suggests a common origin for these two strains.

The outer membrane protein profiles of all strains were identical. These results do not agree with those described by Vaisanen-Rhen *et al.* (1984), who found differences between porins, protein K and OmpA profiles in uropathogenic strains.

The RAPD DNA fingerprinting allowed us to classify the uropathogenic strains into four groups. Most were like strain 63, but even strains which were not uropathogenic such as H10407, HB101 and 055:H6 had an identical profile. We concluded that, at least for the primer used in this work, this technique does not allow differentiating between these types of strains. The use of other primers would be advisable.

Gel electrophoresis of the surface proteins that were expressed at 37°C (but not at 16°C) showed that the strains could be classified into different groups. Even this technique grouped strains that were put into different groups based on the clinical characteristics observed in the patient's urine and in the RAPD-PCR assay.

Other studies on the prevalence and degree of bacterial adherence to uroepithelial cells (Salit *et al.*, 1983; Marild *et al.*, 1988; Sandberg *et al.*, 1988) related that, among *E. coli* strains isolated from patients with cystitis and pyelonephritis, 50 to 60% of strains presented a mean of 20 bacteria/cell and 70 to 100% of strains presented 30 bacteria/cell, respectively, when compared with fecal strains (seven bacteria/cell). Our results agree with these data since the majority (12 of 17) of cystitis-isolated strains presented a mean of 20 bacteria/cell, three presented at least 30 bacteria/cell and two had a mean adherence similar to that shown by fecal isolates. Those strains that had a very high adhesion capacity could have potential to cause pyelonephritis.

Strains (154, 198) that were able to express only type 1 fimbriae and presented the same level of adhesion as strain ORN115 (type 1 producer) showed that the presence of this type of fimbriae permit the bacteria to colonize the urogenital tract.

We concluded that the only characteristic in common to all strains and that could be used as a pathogenicity trait for uropathogenic *E. coli* strains is uroepithelial cell adhesion in the presence of D-mannose, using a positive type 1 fimbria producer strain as control.

We found some cystitis strains (168, 177) that showed a high adherence to uroepithelial cells but did not express mannose-resistant hemagglutination (MRHA) adhesins, and two strains (28, 46) that presented very similar characteristics but adhered to

uroepithelial cells at a different level. These data led us to believe that different virulence factors could be expressed by these strains and studies are underway to investigate them.

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RESUMO

Dezessete linhagens uropatogênicas de *Escherichia coli* causadoras de cistite e uma linhagem causadora de pielonefrite isoladas de pacientes no Hospital das Clínicas da UNICAMP foram estudadas com respeito às suas características de patogenicidade. Para isso, os perfis de proteínas de membrana e de superfície, de plasmídios e "RAPD DNA fingerprinting", produção de colicina e hemolisina e capacidade de adesão a células uroepiteliais foram determinados. Determinou-se que a única característica que pode ser utilizada como padrão de patogenicidade é a capacidade de adesão a células uroepiteliais, visto que todas as demais apresentaram variação entre as diferentes linhagens.

REFERENCES

- Arthur, M., Johnson, C.E., Rubin, R.H., Arbeit, R.D., Campanelli, C., Kim, C., Steinback, S., Agarwal, M., Wilsinson, R. and Goldstein, R. (1989). Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli*. *Infect. Immun.* 57: 303-313.
- Azevedo, J.L. and da Costa, S.O.P. (1973). In: *Exercícios Práticos de Genética*. Companhia Editora Nacional, Editora da Universidade de São Paulo, São Paulo, SP, pp. 171-174.
- Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res.* 7: 1531-1532.
- Blanco, J., Alonso, M.P., Blanco, M., Blanco, J.E., González, E.A. and Garabal, J.I. (1992). Establishment of three categories of P-fimbriated *Escherichia coli* strains that show different toxic phenotypes and belong to particular O serogroups. *FEMS Microbiol. Letters* 99: 131-136.
- Blum, H., Beier, H. and Gross, H.J. (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* 8: 93-99.

- Bolivar, F. and Backman, K.** (1979). Plasmids of *Escherichia coli* as cloning vectors. *Methods Enzymol.* 68: 245-267.
- Boyer, H.W. and Roulland-Dussoix, D.** (1969). A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* 41: 459-472.
- Cavaliere, S.J. and Snyder, I.S.** (1982). Cytotoxic activity of partially purified *Escherichia coli* alpha haemolysin. *J. Med. Microbiol.* 15: 11-21.
- Czirok, E., Milch, H., Csiszar, K. and Csik, M.** (1986). Virulence factors of *Escherichia coli*. *Acta Microbiol. Hung.* 33: 69-83.
- Eisenstein, B.I. and Jones, G.W.** (1988). The spectrum of infections and pathogenic mechanisms of *Escherichia coli*. *Adv. Intern. Med.* 33: 231-252.
- Evans, D.G., Silver, R.P., Evans, D.J., Chase, D.G. and Gorbach, S.L.** (1975). Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for human. *Infect. Immun.* 12: 656-667.
- Evans, D.G., Evans, D.J., Tjoa, W.S. and DuPont, H.L.** (1978). Detection and characterization of colonization factor of enterotoxigenic *Escherichia coli* isolated from adults with diarrhea. *Infect. Immun.* 19: 727-738.
- Evans, D.J., Evans, D.G. and DuPont, H.L.** (1979). Hemagglutination patterns of enterotoxigenic *Escherichia coli* determined with human, bovine, chicken and guinea-pig erythrocytes in the presence and absence of mannose. *Infect. Immun.* 23: 336-346.
- Evans, D.J., Evans, D.G., Hohne, C., Noble, M.A., Haldane, E.V., Lior, H. and Young, L.S.** (1981). Hemolysin and K antigens in relation to serotype and hemagglutination type of *Escherichia coli* isolated from extraintestinal infections. *J. Clin. Microbiol.* 13: 171-178.
- Hacker, J., Hof, H., Emody, L. and Goebel, W.** (1986). Influence of cloned *Escherichia coli* hemolysin genes, S-fimbriae and serum resistance on pathogenicity in different animal models. *Microb. Pathog.* 1: 533-547.
- Hanahan, D.** (1983). Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* 166: 557-580.
- Harber, M.J., Topley, N. and Asscher, A.W.** (1986). Virulence factors of urinary pathogens. *Clin. Sci.* 70: 531-538.
- High, N.J., Hales, B.A., Jann, K. and Boulnois, G.J.** (1988). A block of urovirulence genes encoding multiple fimbriae and hemolysin in *Escherichia coli* O4:K12:H⁻. *Infect. Immun.* 56: 513-517.
- Hofer Scientific Instruments** (1990). *Hofer Electrophoresis Catalog and Exercises (1990-1991)*. San Francisco, USA, pp. 128-168.
- Hughes, C., Phillips, R. and Roberts, A.P.** (1982). Serum resistance among *Escherichia coli* strains causing urinary tract infection in relation to O type and the carriage of hemolysin, colicin, and antibiotic resistance determinants. *Infect. Immun.* 35: 270-275.
- Hughes, C., Hacker, J., Roberts, A. and Goebel, W.** (1983). Hemolysin production as a virulence marker in symptomatic and asymptomatic urinary tract infection caused by *Escherichia coli*. *Infect. Immun.* 39: 546-551.
- Johnson, J.R.** (1991). Virulence factors in *Escherichia coli* urinary tract infection. *Clin. Microbiol. Rev.* 4: 80-128.
- Johnson, J.R. and Stamm, W.E.** (1989). Urinary tract infection in women: diagnosis and therapy. *Ann. Intern. Med.* 111: 906-917.
- Laemmli, U.K.** (1970). Cleavage of structural genes during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Levine, M.M.** (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive and enteroadherent. *J. Infect. Dis.* 155: 337-389.
- Lipsky, B.A.** (1989). Urinary tract infections in men. Epidemiology, pathophysiology, diagnosis, and treatment. *Ann. Intern. Med.* 110: 138-150.
- Low, D., David, V., Lark, D., Schoolnik, G. and Falkow, S.** (1984). Gene clusters governing the production of hemolysin and mannose-resistant hemagglutination are closely linked in *Escherichia coli* serotype O4 and O6 isolates from urinary tract infection. *Infect. Immun.* 43: 353-358.
- MacKenzie, W.R. and O'Hanley, P.** (1991). Recent advances related to virulence and host factors in urinary tract infections. *Curr. Opin. Infect. Dis.* 4: 31-36.
- Marild, S.B., Wettergrem, B., Hellstrom, M., Jodal, U., Lincoln, K., Orskov, I., Orskov, F. and Svanborg Eden, C.** (1988). Bacterial virulence and inflammatory response in infants with febrile urinary tract infection of screening bacteriuria. *J. Pediatr.* 112: 348-354.
- Orndorff, P.E., Spears, P.A., Schauer, D. and Falkow, S.** (1985). Two models of control of *pilA*, the gene encoding type 1 pili in *Escherichia coli*. *J. Bacteriol.* 164: 321-330.
- Orskov, I. and Orskov, F.** (1985). *Escherichia coli* in extra-intestinal infections. *J. Hyg.* 95: 551-575.
- Orskov, I., Williams, P.H., Svanborg Eden, C. and Orskov, F.** (1989). Assessment of biological and colony hybridization assays for detection of the aerobactin system in *Escherichia coli* from urinary tract infections. *Med. Microbiol. Immunol.* 178: 143-148.
- Reid, G. and Sobel, J.D.** (1987). Bacterial adherence in the pathogenesis of urinary tract infections: a review. *Rev. Infect. Dis.* 9: 470-487.
- Salit, I.E., Vavougiou, J. and Hofmann, T.** (1983). Isolation and characterization of *Escherichia coli* pili from diverse clinical sources. *Infect. Immun.* 42: 755-762.
- Sambrook, J., Fritsh, E.F. and Maniatis, T.** (1989). *Molecular Cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Sandberg, T., Kaijser, B., Lidin-Janson, G., Lincoln, K., Orskov, F., Orskov, I., Stockland, E. and Svanborg Eden, C.** (1988). Virulence of *Escherichia coli* in relation to host factors in women with symptomatic urinary tract infection. *J. Clin. Microbiol.* 25: 1471-1476.
- Silveira, W.D., Fantinatti, F. and Castro, A.F.P.** (1994). Transposon mutagenesis and membrane protein studies in an avian colisepticaemic *Escherichia coli* strain. *Braz. J. Genet.* 17: 9-14.
- Sperandio, V. and Silveira, W.D.** (1993). Comparison between enterotoxigenic *Escherichia coli* strains expressing "F42", F41 and K99 colonization factors. *Microbiol. Immunol.* 37: 869-875.

- Svanborg-Eden, C.** (1986). Bacterial adherence in urinary tract infections caused by *Escherichia coli*. *Scand. J. Urol. Nephrol.* 20: 81-88.
- Svanborg-Eden, C., Anderson, B., Aniansson, G., Jodal, U., Lomberg, H., Linder, H. and de Man, P.** (1988). Bacterial adherence in urinary and respiratory tract infection. *J. Jpn. Assoc. Infect. Dis.* 62: 136-148.
- Vaisanen-Rhen, V., Elo, J., Vaisanen, E., Shtonen, A., Orskov, I., Orskov, F., Svenson, S.B., Makela, P.H. and Korhonen, T.K.** (1984). P-fimbriated clones among uropathogenic *Escherichia coli* strains. *Infect. Immun.* 43: 149-155.
- Willians, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V.** (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.
- Young, R.A. and Davis, R.W.** (1983). Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci.* 80: 1194-1198.

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