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Escherichia coli in extra-intestinal infections

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INTRODUCTION

When Theodor Escherich (1885*a*, *b*) first described *Escherichia coli* he looked on it as a saprophytic organism. Soon several investigators found that colibacteria could be isolated from intestinal infections and from many infections outside the intestine, like urinary tract infections (UTI), cholecystitis, wound infections, meningitis, septicaemia, pulmonary infections, and many more. Uhlenhuth (1897) showed that coli strains from pathological processes were more pathogenic in animal experiments than strains isolated from the normal intestine. Smith (1927), who examined strains from white scours in calves, showed that spontaneous acapsular mutants could be obtained from certain colibacteria, and that such mutants were less virulent when injected intra-peritoneally into guinea-pigs.

Several attempts to define toxins in coli strains were also carried out. Interest was centred on skin necrotizing toxins and on haemolysins. Most investigators agreed that it was not possible to isolate the toxins and that the haemolytic and skin-necrotizing abilities were only found in close association with actively dividing bacteria.

Several investigators in the first three decades of this century tried to classify *E. coli*, hoping to be able to define certain strains or serotypes as virulent. However, these attempts did not lead to any conclusive results.

In the 1940s Kauffmann (1943, 1944*a*, *b*, 1947) applied to *E. coli* the serological techniques that he and other investigators had developed for typing of salmonella strains. He showed that coli strains had O antigens that in their serological qualities were very similar to those found in salmonella and, more importantly, that many strains isolated from pathological processes outside the intestinal tract had surface antigens that would inhibit agglutination of the live culture in a so-called O antiserum, i.e. an antiserum produced by immunization of rabbits with boiled culture. He called such antigens L antigens (Kauffmann, 1943). He was now able to establish the first *E. coli* typing scheme (Kauffmann, 1944*a*). Knipschildt (1945) and Vahlne (1945), both close pupils of Kauffmann, developed and refined the typing principles by extension of the number of O groups and by describing two new varieties of surface antigens called A and B antigens, both of which could inhibit O agglutination (Knipschildt, 1945). At that time the chemical nature of the L, A and B antigens was not known; however, it was decided to name them collectively as K antigens (Kauffmann & Vahlne, 1945). For more details about the history, serology and immunochemical nature of *E. coli* K antigens see Ørskov *et al.* (1977) and Jann & Jann (1983).

Kauffmann and his early group of collaborators were, however, also highly interested in the pathogenic properties of *E. coli* in extra-intestinal diseases, and in 1947 he could sum up his own results together with those of Knipschildt (1945), Ewertsen (1946), Vahlne (1945) and Sjöstedt (1946) in this way: 'The results show consistently that certain serological types belonging to the frequent O groups possess a particular pathogenicity for man and animals. This applies especially to O groups 1, 2, 4, 6, 8 and 9. Haemolytic and necrotizing strains are of frequent occurrence with O groups 2, 4 and 6, on which account especially group 6 comprises highly toxic strains. The toxicity of the strains depends on the type (read O:K:H type) of the strain and it is a constant character' (Kauffmann, 1947). When Kauffmann and his collaborators described the traits that might differentiate pathogenic *E. coli* from non-pathogenic ones they compared two groups of strains, one which comprised strains from all types of extra-intestinal infections and one which came from the faeces of healthy individuals. It should be remembered that at that time the association between certain strains and intestinal disease (EPEC, ETEC and EIEC) had not yet been described. Investigations in the years since these early examinations have, however, shown that three groups of extra-intestinal coli infections (UTI, neonatal meningitis and non-neonatal septicaemia) need to be treated separately even though several traits and virulence factors may be found in strains isolated from all extra-intestinal diseases.

In dealing with the activities of *E. coli* outside the intestinal tract we have in this review paper, quite appropriately for a bacterial baptismal centenary, paid most attention to the bacterium *Escherichia coli*, and only in a few places have we given details from the many investigations that describe the response of the host.

URINARY TRACT INFECTIONS (UTI)

E. coli is the most common urinary pathogen found in non-hospitalized patients of all ages. In general UTI is diagnosed by demonstration of $\geqq 10^5$ bacteria per ml of midstream urine or by any number of bacteria from urine obtained by suprapubic bladder puncture. Recently it has been proposed to use a lower limit than 10^5 bacteria per ml for the demonstration of UTI (Stamm *et al.* 1982). Some cases of bacteriuria are asymptomatic (ABU), some are limited to the bladder or lower urinary tract (cystitis), and some involve the upper tract, i.e. the pelvis and the kidneys (pyelonephritis).

Recurrent UTI is not uncommon; it can be caused by the *E. coli* strain that caused the first attack but which was only temporarily suppressed (relapse) or by an unrelated strain (reinfection). Abnormalities of the urine flow caused by malformations, stones, residual urine and vesico-ureteric reflux predispose for recurrent UTI, but such abnormalities cannot always be demonstrated in recurrent UTI.

Several reports on *E. coli* from UTI do not distinguish between these different infections. This is stressed because the coli strains isolated from each of these types of UTI show certain characters or virulence factors with varying prevalence.

It is generally accepted that UTIs are usually ascending infections caused by bacteria originating in the intestinal tract, because a high similarity exists between

E. coli strains from urine and faeces of infected individuals (Turck, Petersdorf & Furnier, 1962; Vosti *et al.* 1964; Grüneberg, 1969). The infecting *E. coli* type can often be isolated from the faeces before or at onset of symptomatic UTI (Grüneberg, Leigh & Brumfitt, 1968; Lidin-Janson *et al.* 1977), and it has furthermore been shown that colonization of the vaginal introitus and the periurethral area precedes the infection (Cooke & Ewins, 1975; Bollgren & Winberg, 1976; Fowler & Stamey, 1977).

Two theories have been presented to explain the emergence of UTI with a faecal strain: (1) the prevalence theory which says that the urinary strain has dominated the faecal flora, and (2) the special pathogenicity theory which proposes that certain virulence factors have to be present in strains able to invade the urinary tract (Grüneberg, Leigh & Brumfitt, 1968). The two theories are probably not contradictory, but the correctness of the prevalence theory is difficult to prove because of the lack of prospective studies. However, from studies carried out in recent years it has appeared that *E. coli* isolated from non-obstructive cases of acute pyelonephritis without reflux are characteristically endowed with certain traits considered of importance for virulence. The traits are: certain O groups; certain K antigens; amount of K antigen; certain adhesins; haemolysin production; resistance to serum bacteridical activity; resistance to phagocytosis; siderophore production.

O antigens

E. coli strains of a relative small number of O groups, namely O1, O2, O4, O6, O7, O18 and O75, have been reported to account for a major part of O-groupable UTI strains from different parts of the world (Ranz, 1962; Turck, Petersdorf & Furnier, 1962; Kunin, Deutscher & Paquin, 1964; Vosti *et al.* 1964; McGeachie, 1965; Ørskov & Ørskov, 1967; Grüneberg, Leigh & Brumfitt, 1968). It has also been demonstrated that strains from the first infection showed a greater concentration of these O groups compared with strains from recurrent infections (McGeachie, 1965: Bergström *et al.* 1967; Ørskov & Ørskov, 1967; Mabeck, Ørskov & Ørskov, 1971*b*; Sietzen, 1979). When UTIs were diagnosed as ABU, cystitis or pyelonephritis, it was furthermore demonstrated that strains of the above-mentioned O groups with the addition of O16 were found in ~ 80% of pyelonephritis, in ~ 59% of cystitis and in 31% of ABU strains (Lindberg *et al.* 1975*a*). In addition, strains from pyelonephritis without reflux more frequently belong to these O groups than do strains from pyelonephritis with reflux (Lomberg *et al.* 1984; Svanborg Edén *et al.* 1985). No particular toxicity of these O antigens compared with other O antigens, i.e. these lipopolysaccharides as such, has ever been shown; but it has been shown by comparing isogenic $O75^+$ K5 and $O75^-$ K5 isolates that presence of O antigen contributes to the ability to persist in the mouse kidneys and bladders (Hagberg *et al.* 1984).

It has been reported that spontaneously agglutinating strains (rough) were particularly frequent among ABU strains (Olling *et al.* 1973; Lindberg *et al.* 1975*a*), and that O antigens of strains from acute pyelonephritis and ABU belonging to the same O groups behaved differently, e.g. strains from acute pyelonephritis absorbed O antibodies in rabbit O antisera better than did strains from cases of ABU (Lindberg *et al.* 1975*b*). A change from O group typability to spontaneous

agglutination in successive UTI isolates has also been observed (Bettelheim & Taylor, 1969; Lindberg *et al.* 1975*b*; Webb, Goodwin & Green, 1982). These findings have been explained by a loss of full-length O polysaccharide chains of the lipopolysaccharides in patchwise areas of the bacterial surface (Webb, Goodwin & Green, 1982). As pointed out by Hanson *et al.* (1977), such changes may be an example of 'antigenic drift' induced by antibodies of the host as noted for *Vibrio cholerae* in mice (Sack & Miller, 1969).

K antigens

Vahlne (1945) and Sjöstedt (1946) showed that presence of K antigen was more common in UTI strains than in faecal strains. The most frequently found types are K1, K2, K3, K5, K12 and K13 (Mabeck, Ørskov & Ørskov, 1971*a*; Kaijser *et al.* 1977; Ørskov *et al.* 1982*a*). Glynn & Howard (1970) and McCabe *et al.* (1975) demonstrated that the K antigen was present in larger quantities in urinary strains than in strains from faeces; strains from the upper urinary tract were particularly rich in K antigen (Glynn, Brumfitt & Howard, 1971; Brooks *et al.* 1980). The K antigens were not serologically typed. The amount of K antigen was measured by the ability of culture supernatant to inhibit the agglutination of sheep red blood cells by antiserum. Kaijser (1973) measured the amount of K antigens K1, K2 and K13, both by the haemagglutination-inhibition technique and by crossed immunoelectrophoresis, and provided additional support for the finding that K antigen is present significantly more often and in greater amounts among *E. coli* from cases of pyelonephritis than among strains from cystitis or from stools of healthy children. In this case both O and K antigens were determined, and pyelonephritis and cystitis strains of the same O:K type differed in K antigen amount. Strains rich in K antigen were less sensitive than others to phagocytosis as well as to bactericidal killing (Glynn & Howard, 1970).

Kétyi, Naumann & Nimmich (1983) inoculated several rough *E. coli* strains with or without the K1 antigen directly into the bladder (i.e. through the abdominal wall) of suckling mice (Kétyi, 1981). The strains were not isogenic. After 14–24 days the bladder and kidney infections were estimated. Possession of the K1 antigen was shown to have a virulence-enhancing effect. By comparing different K^+ strains with their K^- mutants Verweig-van Vught *et al.* (1983) found that K antigens were of minor importance for mouse nephropathogenicity. They only examined one $K1^+$ strain, and this was not nephropathogenic but was able to cause a more general infection (termed group III strains), and this ability was lost by the K^- mutant. However, in the model used by Verweig-van Vught *et al.* (1983) the bacteria were injected intravenously, and viable counts of the kidneys were performed 8 h after injection (van den Bosch, de Graaf & MacLaren, 1979). In contrast to this model, that of Kétyi (1981) was designed to bring about an ascending UTI.

O:K:H serotypes

Mabeck, Ørskov & Ørskov (1971*a*) found that symptoms of acute pyelonephritis were not correlated with particular H antigens, but that some *E. coli* serotypes, namely O2:K1:H4, O4:K12:H5 and O6:K2ac:H1, were associated with a disproportionately high frequency of acute pyelonephritis. Ørskov *et al.* (1982*b*)

reported that strains of serotypes O1:K1:H7, O4:K12:H5, O6:K2:H1 and O16:K1:H6 were more frequently associated with pyelonephritis than with cystitis, while the opposite was true for strains of serotype O6:K13:H1, which was therefore considered as a cystitic type. Väisänen-Rhen *et al.* (1984) found that 57% of O antigen-typable strains from pyelonephritis belonged to the following six serotypes: O1:K1:H7, O4:K12:H1, O4:K12:H1, O6:K2:H1, O16:K1:H6 and O18ac:K5:H7; the serotype O6:K13:H1 was also here associated with cystitis.

Haemolysin

In 1921 Dudgeon, Wordley & Bawtree pointed out that haemolytic strains are more common among UTI strains than among strains from the faecal flora. This fact was later confirmed by several authors, e.g. Cooke & Ewins (1975), Minshew *et al.* (1978) and Hacker *et al.* (1983). In addition, the percentage of haemolytic strains has been found to be higher among strains from pyelonephritis than among those from cystitis, ABU or the intestinal tract (Väisänen-Rhen *et al.* 1984). Knipschildt (1945) and Kauffmann (1947) demonstrated that haemolytic *E. coli* mainly belonged to strains of O groups 2, 4 and 6, and UTI strains are frequently of these O groups or of O groups 18 and 75, which are also often haemolytic (Cooke & Ewins, 1975; Hughes, Phillips & Roberts, 1982). However, strains of some O:K:H serotypes within these O groups are more often haemolytic than others (Vahlne, 1945; Sjöstedt, 1946). Van den Bosch *et al.* (1981) studied the influence on virulence of haemolysin production in the experimental mouse model mentioned above, which involves intravenous injection of the bacteria (van den Bosch, de Graaf & MacLaren, 1979). They concluded that haemolysin production was a decisive virulence factor in most of the mouse nephropathogenic strains, called group II strains, since 6 out of 7 strains became avirulent after elimination of the ability to produce haemolysin. These 6 strains were of O:K serotypes O6:K2, O75:K$^-$, O4:K3 or O139:K82. Transfer of an Hly plasmid to a K12 strain did not render this strain virulent in the same mouse model (Waalwijk *et al.* 1982). Reduction or elimination of the haemolysin production in a group II strain by insertion of transposon Tn5 in the *hly* gene resulted in reduction or loss of nephropathogenicity (Waalwijk & de Graaf, 1983). In the suckling mouse model (Kétyi, 1981), where bacteria are introduced via the bladder, Kétyi, Naumann & Nimmich (1983) found that rough strains with haemolytic activity induced bladder and kidney infections, while rough strains without this activity were non-virulent. Van den Bosch, Emödy & Kéty (1982) compared this model with that involving intravenous ingestion of bacteria and found that haemolysin played a role in group II strains no matter whether infection occurred via the bladder or intravenously.

As to the importance of haemolysin in strains given intravenously, Smith (1963) found that preparations containing high concentrations of haemolysin were toxic for mice. Emödy *et al.* (1980) could not demonstrate a substantial difference between cultures producing haemolysin and their non-haemolytic derivatives in mice, and van den Bosch *et al.* (1981) also reported that haemolysin was not an important virulence factor in the extremely virulent group III strains which had a more general virulence for mice.

When haemolytic strains are given intraperitoneally (i.p.) or intranasally to

mice, they are more toxic than non-haemolytic strains (Smith & Linggood, 1971; Kéty *et al.* 1978; Emödy *et al.* 1980). Welch *et al.* (1981) compared the virulence of a strain into which the *hly* genes had been cloned with that of the same strain which had become Hly^- after insertion of a transposon. When inoculated i.p. into rats, the Hly^+ strain was more virulent than the Hly^- mutant. In a recent paper Smith & Huggins (1985) showed that Hly^+ bacteria given i.p. to mice multiply and produce haemolysin, and when sufficient haemolysin has been liberated, the animals die. The transposon-inserted Hly^- mutant of the strain also caused death of the mice, but not until the bacteria had multiplied to a much greater extent.

The mechanism and site of action of haemolysin is not yet known. Partially purified preparations of haemolysin from *E. coli* strains are cytotoxic for human leucocytes and for fibroblasts *in vitro* (Cavalieri & Snyder, 1982*a–c*). Gadeberg, Ørskov & Rhodes (1983) described a cell-associated toxic effect for human monocytes and granulocytes in haemolytic *E. coli* strains; this effect was not inhibited by autologous plasma, in contrast to the effect produced by haemolysin-containing culture supernatants. Isogenic Hly^- bacteria were not cytotoxic (Gadeberg & Ørskov, 1984). Thus a haemolytic *E. coli* culture may be virulent because of a toxic effect on various cells, and in addition this effect may be strengthened because the growth of the strain is stimulated by iron made available by lysis of the erythrocytes (Linggood & Ingram, 1982; Waalwijk, MacLaren & de Graaf, 1983). See also aerobactin under the section on bacteraemia.

Serum resistance

It has been claimed that the ability to resist the bactericidal activity of serum was present more often in pyelonephritis *E. coli* strains than in ABU strains (Gower *et al.* 1972; Olling *et al.* 1973; Björkstén & Kaijser, 1978), and that it was significantly correlated with haemolysin production and with O types O4, O6, O18 and O75 (Hughes, Phillips & Roberts, 1982). For further information on serum resistance see under bacteraemia.

Phagocytosis

E. coli with MS fimbriae/adhesins bind to and activate human polymorphonuclear leucocytes (PMV) (Bar-Sharit *et al.* 1977; Mangan & Snyder, 1979; Silverblatt, Dreyer & Schauer, 1979), while strains with MR fimbriae of the gal-gal recognizing type are poor in this respect (Blumenstock & Jann, 1982; Svanborg Edén *et al.* 1984); this means that possession of these last-mentioned fimbriae may confer resistance to phagocytosis. For other information on phagocytosis see under bacteraemia.

Adhesion and fimbriae

Svanborg Edén *et al.* (1976, 1978) have shown that *E. coli* from cases of acute pyelonephritis in children adhere to human urinary epithelial cells to a greater extent than strains from cases of cystitis, and these latter strains adhere in higher number than strains from ABU. It was further demonstrated that the ability to adhere was strongly correlated with the presence of fimbriae and mannose-sensitive agglutination of guinea-pig erythrocytes; however, the adhesion to urinary epithelial cells was not inhibited by D-mannose, suggesting that this adhesion

might be mediated by some bacterial surface factor co-appearing with the fimbriae (Svanborg Edén & Hansson, 1978). Källenius & Möllby (1979) reported that adhesion to human periurethral cells correlated to mannose-resistant agglutination of human erythrocytes. A very high proportion of strains with those two abilities were present among acute pyelonephritis isolates from children, in contrast to the proportion among normal faecal *E. coli* as shown by Hagberg *et al.* (1981). The attachment is mediated by a specific binding to glycolipid receptors of the globoseries (Leffler & Svanborg Edén, 1981), and fimbriae binding in this way have been termed GS fimbriae (Svanborg Edén *et al.* 1983*a*). The receptor is part of the P blood group antigen containing α-D-galactose-(β1-4)-D-galactose residues, and therefore the fimbriae have also been termed P fimbriae (Källenius *et al.* 1980, 1981). Another name is pap pili (pyelonephritis-associated pili) (Hull *et al.* 1981) or gal-gal pili (O'Hanley *et al.* 1985).

The view that gal-gal fimbriae are virulence factors in UTI has its opponents, however. Thus Harber *et al.* (1982) questioned the role played by fimbriae, since bacteria freshly shed in the urine of infected patients failed to bind to epithelial cells. As pointed out by Kunin (1982) and Svanborg Edén *et al.* (1982) an explanation might be that the patients in the series of Harber *et al.* (1982) had complex urologic problems, in which cases the bacteria do not need adhesive properties. This suggestion was strongly supported by the findings of Lomberg *et al.* (1984), who demonstrated a lower frequency of adhesiveness among strains from children with reflux than among those without reflux. Svanborg Edén *et al.* (1982) further suggested that the findings of Harber *et al.* (1982) might be due to the fact that not all bacteria in a culture attach to uroepithelial cells. It is therefore understandable that UTI patients shed non-adhering bacteria. The question has been raised why not all bacteria in a fimbriated culture are fimbriated (Ørskov *et al.* 1982*b*). An answer could be phase variation, a mechanism first described for type 1 fimbriae by Brinton, Buzzell & Lauffer (1954). This explanation is supported by the recent report by Rhen, Mäkelä & Korhonen (1983) and Nowicki *et al.* (1984), who showed that an extremely rapid phase variation takes place between different fimbrial types present in an O4:K12 strain.

Receptor cells in adherence tests

In vitro adherence experiments with UTI strains have mainly been carried out with epithelial cells from the sediment of the urine of healthy persons or with periurethral cells from healthy individuals (Svanborg Edén *et al.* 1976; Schaeffer, Amundsen & Schmidt, 1979; Källenius, Möllby & Winberg, 1980). No difference in receptivity between these types of cell has been reported. Buccal epithelial cells have also been used by some workers. Schaeffer *et al.* (1982) described a strong correlation between receptivity of vaginal and buccal cells, while Varian & Cooke (1980) found that UTI strains adhered better to urinary epithelial cells than to buccal cells. UTI strains expressing only gal-gal fimbriae bind to both urinary and buccal cells, although the receptivity of buccal cells may be lower. However, many UTI strains express in addition to gal-gal fimbriae some fimbriae antigenically termed F1C (Ørskov & Ørskov, 1983) with no haemagglutinating ability, and *E. coli* with only these last-mentioned fimbriae confer adherence to buccal cells but not to uroepithelial cells (Ørskov *et al.* 1982*b*). Since a single *E. coli* strain may

express several kinds of fimbriae (Duguid & Old, 1980; Jann, Jann & Schmidt, 1981; Ørskov *et al.* 1982*b*; Korhonen *et al.* 1982), no conclusion about fimbriae and attachment to different epithelial cell types can be drawn until the fimbriae of the strains examined have been analysed.

Serology of MR fimbriae

Fimbriae present in UTI strains causing agglutination of human and monkey erythrocytes in a mannose-resistant manner (MRHA) were examined serologically by means of crossed immunoelectrophoresis (CIE), and different types received the F antigen numbers F7–F13 (Ørskov, Ørskov & Birch-Andersen, 1980; Ørskov *et al.* 1982*a*, Ørskov & Ørskov, 1985) (the F antigen numbering of all fimbriae regardless of morphology and receptor binding was suggested for practical reasons (Ørskov & Ørskov, 1983)).

Test strains for F antigens F7–F13 all agglutinate gal-gal (P) receptor-coated particles, although F10 less well than the others. There is almost no relationship between antigens F7–F13 when any two of those antigens are examined for cross-reaction against one antiserum in CIE. This type of examination was carried out by incorporation of a heterologous F antigen extract in the intermediate gel of each test F antigen CIE system. However, several of the antigens F7–F13 do cross-react when examined by another method in which a heterologous F antiserum is added to the intermediate gel instead of an F antigen extract, i.e. when one antigen is tested against two antisera (Ørskov & Ørskov, 1983, 1985). Chemically these as well as the type 1 (see below) fimbrial antigens constitute a group of evolutionarily related proteins (Klemm, Ørskov & Ørskov, 1983).

Correlation of F antigen and O:K:H serotype

By examination of the F antigens found in strains of the most common O:K:H serotypes among cystitis and pyelonephritis strains causing MRHA of human erythrocytes (Ørskov *et al.* 1982*b*; Ørskov & Ørskov, 1983, 1985) an association was found between F antigen and O:K:H serotypes, a finding which was suggested to reflect nature's selection of certain virulent clones rather than genetic linkage of different structures (Ørskov *et al.* 1982*a*). The O:K:H:F types are the following: O1:K:H$^-$:F, O1:K1:H7:F11, O2:K1:H4:F11, O4:K12:H1:F11, F12 related, O4:K12:H5:F13, F12 related and Fy (not yet characterized), O6:K2:H1:F7 ($F7_1$ and/or $F7_2$), O7:K1:H$^-$:F10 and O16:K1:H6:F12, some in addition F11.

F antigen 1C which does not cause haemagglutination is present in many UTI strains, but has been left out from the O:K:H:F serotype formula given here.

It is noteworthy that the typical cystitis serotype O6:K13:H1 does not have MRHA activity (Ørskov *et al.* 1982*b*).

E. coli serotype O6:K5:H1 are among strains isolated from cystitis or pyelitis. Some of these strains adhere to uroepithelial cells, but the adhesin has not yet been classified antigenically. Tullus *et al.* (1984) have recently reported that P (gal-gal)-fimbriated O6:K5 strains caused epidemic outbreaks of acute pyelonephritis in children less than 2 years old by nosocomial spread in a neonatal ward.

The MRHA capacity and adherence of strains belonging to the above-mentioned frequent UTI serotypes is ascribed to the gal-gal recognizing fimbriae, but may

in some cases also be due to adhesins not recognizing the gal-gal receptors. This may, for example, be the case in O7:K1:H⁻ strains, in which F10 is not invariably present. The test strain of the F10 antigen is of this O:K:H type and recognizes gal-gal receptor-coated particles, but not so strongly as the test strain of antigens F7, F8, F9, F11, F12 and F13.

It has been reported that strains of O group 2 possess adhesins that recognize the amino-terminal part of glycophorin A on human blood group M erythrocytes (Väisänen *et al.* 1982) and that O2:K1 and O18:K1 strains possess fimbria recognizing sialyl-galactosides (Parkinen *et al.* 1983; Korhonen *et al.* 1984). Such adhesins are occasionally present in UTI strains (Väisänen-Rhen *et al.* 1984) and have been termed M and S fimbriae, respectively (Korhonen *et al.* 1984). No report has appeared regarding the adherence of such strains to urinary epithelial cells. Strains of O group 75 also with MRHA activity for human erythrocytes are not infrequently present among UTI strains (Ørskov & Ørskov, 1982*b*; Väisänen-Rhen *et al.* 1984). A non-P (gal-gal), M or S haemagglutinin provisionally termed O75X, of coil-like structure, has been demonstrated in O75:K5:H- strains isolated from UTI and normal faeces (Väisänen-Rhen, 1984).

Non-fimbrial, non-gal-gal binding adhesins causing MR haemagglutination of human erythrocytes have recently been shown in a pyelonephritic *E. coli* of O group O2 (Labigne-Roussel *et al.* 1984) and in a urinary strain of serotype O83:K1:H4 (Goldhar, Perry & Ofek, 1984). Furthermore a non-gal-gal binding adhesive protein capsule consisting of exceedingly fine filaments was demonstrated in three *E. coli* strains, one of which was an O21:K7:H4 UTI isolate (Ørskov *et al.* 1985). The importance of these adhesins is not known yet.

MS fimbriae (type 1)

Most *E. coli* strains are capable of producing type 1 fimbriae mediating a mannose-reversible (MR) binding capacity (Duguid & Gillies, 1957) and thus also UTI strains with MR adhesins have this capacity. These MS fimbriae bind to monkey kidney cells (Salit & Gotschlich, 1977) and to rat uroepithelial cells (Korhonen, Leffler & Svanborg Edén, 1981). Binding to human uroepithelial cells has been described (Schaeffer, Amundsen & Schmidt, 1979; van den Bosch *et al.* 1981), but it has also been reported that most strains with MSHA adhere poorly to human uroepithelial cells, and that if they attach they attach only to squamous cells, not to transitional cells, in contrast to those with MRHA activity of the gal-gal binding type (Hagberg *et al.* 1981). However, Ørskov, Ørskov & Birch-Andersen (1980) demonstrated that strains with type 1 fimbriae specifically adhere to urinary mucus, which was shown to be identical to Tamm Horsfall protein (Ørskov, Ferenz & Ørskov, 1980), and these authors suggested that trapping of type 1 fimbrial bacteria by the mucus could be considered as a non-immune resistance mechanism. The binding of type 1 fimbriae to urinary mucus (alias Tamm Horsfall protein), has been confirmed by Chick *et al.* (1981).

Role of adhesion for experimental infection

The role of MS and MR (gal-gal) fimbriae for reproduction of disease has been studied in mice, since both kinds of fimbriae attach to mouse uroepithelial cells, while rat cells bind only MS fimbriae (Hagberg *et al.* 1983*a*). About 10^{10} bacteria

were installed into the bladder through a polyethylene catheter. The animals were sacrificed at different times after infection, and viable counts were performed on homogenized bladders and kidneys. The strains used were mutants of an O75 strain isolated from a case of acute pyelonephritis expressing either MS or MR or both kinds of fimbriae, or transformants obtained by cloning genes from strain J96 (O4:K6) coding for either the MS or the MR fimbriae into a normal faecal strain (O16:K1) or into 'K12' (Hagberg *et al.* 1983*b*; Svanborg Edén *et al.* 1983*b*). Presence of MR fimbriae enhanced bacterial recovery from both kidneys and bladders, while mutants and transformants with MS fimbriae were recovered in larger numbers from the bladders than those expressing the MR fimbria, but seemed to give little advantage in the kidney. The transformants derived from both the rough K12 strain and the smooth faecal strain (O16:K1) adhered *in vitro* as well as or better than the mutants derived from a pyelonephritis isolate. Yet the transformants were at a significant disadvantage *in vivo*, i.e. adhesive capacity adds to infectivity in bacteria with other virulence factors but is insufficient to confer virulence as such. Also O'Hanley *et al.* (1985) compared the pathogenic significance of MS and MR (gal-gal) fimbriae in the mouse model and cloned from strain J96 the genetic determinant for these fimbriae into a K12 strain. No colonization of the renal pelvis took place after installation of 10^6 bacteria of the MS clone, while administration of 10^{10}–10^{12} MS bacteria resulted in acute ureteric reflux and colonization of the kidneys. In contrast only 10^6 of the cloned bacteria expressing MR fimbriae colonized the kidneys. Since a titratable factor in the urine identical to Tamm Horsfall protein bound to MS fimbriae, it was suggested that the reason why the MS clone did not mediate renal colonization when inocula $\leqq 10^8$ bacteria were installed, was that the binding capacity for cell-surface receptor molecules present in the mice epithelial cells was nullified by the Tamm Horsfall protein, and this effect could be overcome when larger doses were given.

NEONATAL MENINGITIS: O AND K ANTIGEN

E. coli is a major cause of neonatal meningitis, and *E. coli* meningitis has a high mortality rate and frequency of sequelae (Robbins *et al.* 1974*a*; Mulder & Zanen, 1984). Robbins *et al.* (1974*a*), McCracken *et al.* (1974) and Sarff *et al.* (1975) showed that 84% of *E. coli* isolated all over the U.S.A. from patients with this disease carried the K1 antigen. Similar high frequencies of K1-carrying strains have been found in Europe (Cheasty, Gross & Rowe, 1977; Mulder & Zanen, 1984; Mulder, van Alphen & Zanen, 1984). K1 strains are found frequently but with lower frequencies in faeces of infants and adults (Robbins *et al.* 1974*a*; Sarff *et al.* 1975; Ørskov & Ørskov, 1975). The most frequent O groups associated with K1 among American strains were O7, O18, O1 and O6 (Robbins *et al.* 1974*a*; McCracken *et al.* 1974), and the same O groups are found in European strains from neonatal meningitis. However, at least one O group, O83, which is highly prevalent in continental Europe (Handrück *et al.* 1982; Mulder, van Alphen & Zanen, 1984), has only rarely been found in the U.S.A. Based on these results, it was natural to call the K1 antigen a virulence factor. The K1 antigen and the capsular polysaccharide of *Neisseria meningitidis* type B are chemically and immunologically

very similar (Grados & Ewing, 1970; Robbins *et al.* 1974*b*): both are linear homopolymers of an α 2–8 linked *N*-acetyl neuraminic acid (NANA) (Barry & Goebel, 1957; Bolanos & de Witt, 1966). The K1 antigen is a poor immunogen in rabbits when injected intravenously as live organisms. High-titred capsular antisera can, however, be obtained with formalinized *N. meningitidis* group B organisms by repeated immunization of horses, and antiserum from one horse (no. 46) is used currently in many laboratories as an anti-K1 serum. The antiserum agar technique (Sarff *et al.* 1975) that has been used widely to screen for *E. coli* K1 is based on the finding that a precipitation halo will develop around colonies when K1 cultures are grown on K1 antiserum-containing plates. Gross, Cheasty & Rowe (1977) have isolated a small series of K1-specific phages that are used in many laboratories for K1 detection. Practically all K1 cultures will give rise to colonies of two forms determined by the presence of O-acetylated (OAc^+) or non-O-acetylated (OAc^-) K1 polysaccharide. Either type of colony will give rise to colonies of both types when subcultured (Ørskov *et al.* 1971, 1979). This type of variation was called form variation, in analogy to the form variation described in salmonella O antigens (Kauffmann, 1940). The OAc^+ K1 polysaccharide is resistant to hydrolysis by neuraminidases (Ørskov *et al.* 1979) and is more immunogenic in rabbits than the OAc^- polysaccharide. Most cultures from neonatal meningitis and from other sources are found predominantly in the OAc^- state when first examined; at present, however, no conclusion can be drawn about the possible influence of form variation in natural disease. The genetic background for the form variation phenomenon has been investigated by Silver, Vann & Aronson (1984).

The occurrence of the same capsular polysaccharide in two different pathogens causing bacterial meningitis, the coli K1 antigen and the group B antigen of *N. meningitidis*, has led to speculation about the importance of such cross-reacting antigens in 'natural immunity' to meningococcus B disease (Robbins *et al.* 1974*b*). The common occurrence of K1 strains in the healthy intestine speaks for this possibility, while the poor immunogenicity of the K1 antigen speaks against it (Robbins *et al.* 1974*b*; Ørskov *et al.* 1979). Another pair of such cross-reacting antigens is the *Haemophilus influenzae* type b capsule and the coli K100 antigen that are both polyribitol phosphates (Schneerson *et al.* 1972; Robbins *et al.* 1976). *E. coli* O75:K100 fed to adult volunteers caused significant rises in anti-capsular antibodies, and the not infrequent occurrence of *E. coli* with the K100 antigen might well explain the occurrence of antibodies to *H. influenzae* type b antigen in the adult population (Robbins *et al.* 1973). Other examples of antigenic cross-reactions between coli K antigens and the capsular antigens of invasive bacteria have been described by Robbins *et al.* (1974*b*).

BACTERAEMIA

Neonatal meningitis with *E. coli* is most likely preceded by a bacteraemic phase, and several investigators count strains from neonatal meningitis and bacteraemia as one group. *E. coli* septicaemia and bacteraemia do not occur only in neonates, and most published bacteraemia materials contain strains from all age groups. The

strains described in such studies arrived in the blood from many different sites of infection; especially frequently, however, from the urinary tract (Kreger *et al.* 1980). Many strains came from immunocompromised patients.

O antigens

Several groups of investigators have examined bacteraemia strains for their O antigens. Vosti *et al.* (1964) found O groups O6, O4, O1, O75, O50, O16, O7, O21, O17 and O18, in that order of frequency. Maiztegui *et al.* (1965) presented a very similar list of frequent O groups. McCabe *et al.* (1978) found in another large body of US data that O groups O6, O4, O2, O16, O18 and O7 were found frequently in both adult and infant bacteraemia, and that no significant difference could be found between the occurrence of the single O groups in the two groups of patients. Ørskov & Ørskov (1975), who examined 559 strains from different places in Denmark, found the following order of frequency: O2, O4, O6, O75, O9, O8, O18, O7, O22 and O1. Antisera corresponding to the most common O groups could classify 57% of the strains. Cross *et al.* (1984), who examined a large collection of material from Washington D.C., found that O groups O6, O4, O1, O18ac, O16, O75, O8, O2, O12 and O15 were the most frequent ones and made up 59% of all strains. Thus, even though minor differences among these materials from various geographical areas could be found, the uniformity of results is impressing. Most of these O groups are also found frequently in other types of extra-intestinal infections, but in addition in the normal healthy intestine – much more rarely however – and one can therefore not conclude from these results that some O group strains have special invasive capacities. This 'special pathogenicity' versus 'prevalence'discussion has already been treated above (in the UTI section).

K antigens and O:K:H serotypes

McCabe *et al.* (1978) also carried out K determination on their strains and found K antigen in the following order of frequency: K1, K2, K12, K3, K13, K15, K8 and K52. Because of lack of a useful antiserum, K5 determination was not carried out. Among strains that could be typed for both O and K antigen they found the following O:K combinations to be the most common: O16:H1, O6:K2, O1:K1, O18:K1, O4:K12 and O7:K1. We have recently examined the above-mentioned Danish material in more detail (Ørskov & Ørskov, in preparation) and found that the most frequent K antigens were: K1, K12, K5, K13, K2, K15, K52 and K25, in that order. Amongst 363 O groupable strains 156 O:K:H serotypes were found; 97 of these were represented by only one strain, whilst some serotypes were represented by many strains: O4:K12:H5 by 28 strains, O2:K1:H4 by 14 and O6:K15:H31 by 8 strains. Some of these frequent O:K:H serotypes found in this Danish material have also been found among blood isolates in other locations. Evans *et al.* (1981), who examined a large number of blood isolates with special attention to the association between haemagglutination, haemolysin production and serotype, also found these O:K:H serotypes well-represented. Korhonen *et al.* (1985) found that O18ac:K1:H7, O7:K1:H1, O6:K2:H1 and Rough:K1:H33 altogether counted for 33% of 63 Finnish strains from infants blood. Cross *et al.* (1984) found O18ac:K1, O1:K1 and O16:K1 to be the most common O:K types in the above-mentioned material from a Washington DC hospital. Thus, even

though complete O:K:H serotyping has not been published from all studies based on American strains, it is most likely that it really is the same serotypes that are found frequently in blood in USA and in Scandinavia; O16:K1 may be an exception, being highly prevalent in American blood isolates and not at all represented among the published results based upon Scandinavian strains. As we know that O16:K1 strains can be found in Denmark (data not published), we can only guess if this difference is based on an unexplained different prevalence in the intestine of the general population or if the US O16:K1 serotypes carry special virulence factors. When discussing serotype distribution in different geographical locations, one could mention that Mabeck, Ørskov & Ørskov (1971*a*), who examined UTI strains in a certain area, found unexplained changes in the prevalence of the serotype O2:K1:H4 over a three year period.

Bactericidal serum activity

Sjöstedt (1946), who examined isogenic capsulated and non-capsulated variants of well-defined O:K:H serotypes, noted that in 25% of strains the capsulated forms were the more serum resistant ones. The only two K1 strains examined fell in this group; many strains belonging to O8 and O9 with K antigens of the A type showed no serum resistance. He also compared serum resistance and toxicity measured in LD50 experiments on intraperitoneally infected mice and found that no correlation could be shown between toxicity and serum resistance. He concluded from the examination of many isolates that strains with the same O:K:H serotype had the same resistance to the bactericidal properties of blood. Glynn & Howard (1970) quantitated *E. coli* K antigens by the ability of saline extracts to inhibit the agglutination of sheep erythrocytes in anti-erythrocyte serum. They found that K-rich strains defined by this method were more resistant to serum killing that K-poor ones. McCabe *et al.* (1975, 1978) detected no differences in serum resistance among strains with different K antigens from a collection of blood isolates. Taylor (1976) saw no correlation between susceptibility to serum killing and the amount of K antigen in a series of urinary strains. Van Dijk *et al.* (1979) came to very similar conclusions after examination of blood and faecal strains.

Taylor & Robinson (1980) found that the genetic transfer of the *his* linked genes for the K27 antigen did not change the sensitivity of recipients, and later Opal, Cross & Gemski (1982) confirmed this finding. Based on the above reports it can therefore be concluded that many acidic K antigens have little or no role in protection against serum killing. On K antigen, K1, has a unique position because of its close association with cases of neonatal meningitis (see above). While Olling (1977) and Björkstén *et al.* (1976) could find no simple correlation between occurrence or amount of K1 antigen in clinical isolates, Stevens *et al.* (1978) found that K1 antigen inhibited complement activation. Cross *et al.* (1984), who examined a large collection of strains from blood, reported that 95% of the strains were serum resistant and related this ability to the presence of K1 and other K antigens; K1 was by far the most common K antigen. Many such K1 strains had a rough lipopolysaccharide at the same time; in this respect K1 strains were unique, as a common association between a degraded LPS and other K antigens could not be found. Silver *et al.* (1981) cloned the K1 genes into a K12 host and found that the recipient K12 strain expressing the K1 polysaccharide was resistant to serum

killing. Timmis *et al.* (1981) found in similar cloning experiments that K12 recipients of K1 were resistant to serum killing at low concentrations of serum, but not at higher concentrations. Stevens *et al.* (1978) and Pluschke *et al.* (1983) showed that the sialic acid containing K1 capsule inhibited the activation of the alternative complement pathway. It is probably not possible to draw any definite overall conclusion about the role of acidic capsular polysaccharides in serum killing. The K antigens of the A type, i.e. the electrophoretically slow moving high molecular K antigens (Ørskov *et al.* 1977), which in several respects resemble klebsiella capsular antigens, probably have no major influence on serum killing. The scanty and contradictionary evidence about the electrophoretically fast moving, low molecular K antigens – except K1 – does not allow us to say if some such K antigens may show a similar inhibitory activity.

The role of lipopolysaccharides as a protective factor against serum killing is more thoroughly examined. McCabe *et al.* (1978) found that strains with O2, O4, O6, O7 and O18 were the most serum resistant ones in a large collection from blood. Taylor (1974) described O6, O7 and O18 strains as basically serum resistant, while strains with O1, O2, O4, O9 and O75 were serum sensitive. Henkel (1970) found no correlation between serum resistance and O groups. Taylor (1976) could not detect differences in the amount of extractable lipopolysaccharide from smooth urinary strains that differed in sensitivity to serum. It is, however, a well established fact that smooth to rough mutation, which determines a loss of polysaccharide side chains of the lipopolysaccharide, will also cause a change from serum resistance to serum sensitivity (Rowley, 1968; Taylor, 1975).

Recently Goldman, Joiner & Leive (1984) described a serum-resistant strain of *E. coli*, O111, derived from a serum-sensitive one, that had longer LPS side chains and also a more extensive coverage of the lipid A core subunits, thus supporting the view that the polysaccharide structure and the distribution of LPS, and especially the LPS side chains on the bacterial surface, is of major importance for the sensitivity to the bactericidal forces of serum (Taylor, 1983; Grossman & Leive, 1984). Pluschke & Achtman (1984) have compared serum sensitivity of O18:K1, O7:K1 and O1:K1 strains and related the findings to the diseases from which they were commonly isolated. They pointed out that O18:K1 and O7:K1 strains typically were serum resistant and associated with septicaemia and neonatal meningitis, while O1:K1 strains were usually serum sensitive and rarely found in blood or in cerebrospinal fluid. Serum sensitivity was due to activation of the classical pathway. K1 strains of all three O groups were protected against activation of the alternative complement pathway. In contrast to O7:K1 and O18:K1 strains, O1:K1 strains could be killed by the classical pathway without application of O antibodies; differences in the structure of O antigens are probably partly responsible. Using K1 isolates of the same three O groups, Pluschke *et al.* (1983) found that O7:K1 and O18:K1 were able to cause bacteraemia in newborn rats, while O1:K1 strains could not. Disease and stool isolates did not differ in virulence, confirming Sjöstedt's (1946) old observations.

Taylor & Parton (1976) described an outer membrane protein with influence on serum sensitivity which was only functional in strains with full development of lipopolysaccharide side chains. Plasmid-determined factors with an influence on serum killing have been described by several investigators and have been reviewed

extensively by Taylor (1983). One example is the 25-kd *tra* protein coded for by the R6-5 plasmid found in about 2100 copies per cell in the outer surface of the outer membrane, which will increase the survival in rabbit serum of a moderately serum sensitive *E. coli* strain (Moll, Manning & Timmis, 1980). The modification of serum killing caused by such proteins can most clearly be demonstrated in moderately sensitive strains when using low concentrations of serum. Their existence underlines the multi-factorial nature of the resistance of bacteria to the bactericidal forces of serum.

Phagocytosis

Theobald Smith (1927) demonstrated that calf polymorphonuclear leucocytes were inactive against capsulated *E. coli*, while acapsular variants were readily taken up. Sjöstedt (1946) examined many well-defined O:K:H serotypes and also several sets of isogenic capsulated and non-capsulated *E. coli*. He found some correlation between serotype and phagocytosis and also that the capsulated form was resistant to phagocytosis in contrast to the acapsular variant. Serotypes with well-developed capsules, i.e. strains that gave capsular swelling with K antiserum, were never phagocytosed. Such strains would probably today be found to have K antigens of the A type and O antigens O 8, O 9, O 20 or O 101 (Ørskov *et al.* 1977). Sjöstedt (1946) also found that strains belonging to O 1:K 1:H 1, and especially to O 6:K 13:H^-, would readily undergo phagocytosis; in fact all strains with O 6 were easily phagocytosed, while other serotypes would be phagocytosed to a differing degree. Specific O antisera did not increase phagocytosis, while K antibodies, did, according to Sjöstedt (1946).

Weinstein & Young (1976, 1978) found that K1-containing strains from bacteraemia were relatively more resistant to opsonophagocytosis by normal human plasma and polymorphonuclear leucocytes than non-K 1 strains. Addition of K antibody increased the phagocytosis of K 1 bacteria, and the authors suggested that the low immunogenicity of the K 1 antigen could explain the resistance to phagocytosis in normal plasma. K 1-bearing bacteria from stools showed the same degree of phagocytosis when compared with blood isolates. Stevens, Chu & Young (1980) found that the degree of phagocytosis of K 1 strains was inversely related to the amount of capsular antigen. They described an additional sialic acid containing K 1-related antigen that was found in the greatest amount in strains that were more readily phagocytosed. Bortolussi *et al.* (1979) showed that the K 1 antigen protected the K 1 strains against opsonization by the alternative pathway and further that strains with much cell-associated K antigen were more resistant to phagocytosis and more virulent in newborn rats than strains with little capsular polysaccharide. Cross *et al.* (1984) found similarly that the K 1 antigen protected against opsonophagocytosis by normal human serum and neutrophils, and, in contrast to some earlier observations cited above, they found that most other K antigens did not offer any protection against phagocytosis. Thus at the present time more investigation is needed on the role of coli K antigens – other than K 1 – in phagocytosis.

Haemolysin

The observation that many *E. coli* strains from septicaemia are haemolytic has been made by many investigators: Minchew *et al.* (1978), De Boy, Wachsmuth & Davis (1980), Evans *et al.* (1981), Korhonen *et al.* (1985). For more details about the haemolysins as virulence factors see above under UTI.

Aerobactin

Minchew *et al.* (1978), who showed an association of haemolysin production, haemagglutination of human erythrocytes and virulence for chicken embryos in extra-intestinal *E. coli* isolates, further found that 12% of their blood isolates carried the colicin V plasmid (Col V). Quackenbush & Falkow (1979) found, however, that the common occurrence of the Col V plasmid in invasive *E. coli* did not imply that Col V had any influence on the virulence of such strains. Smith & Huggins (1980) showed that the loss of any of the characters O, K or Col V from an O18:K1:H7 Col V carrying *E. coli* would make the strain less virulent for chickens and mice than the parent form. However, the transfer of any or all of these virulence markers to a K12 strain would only bring minor increases in virulence to the original K12 strain. The loss of virulence of the variants of the O18:K1:H7, Col V strain was caused by a decreased ability to invade the body. Williams (1979) and Warner *et al.* (1981) showed that Col V carrying *E. coli* produced a Col V plasmid-determined siderophore, which was chemically a hydroxymate compound identical to aerobactin, originally described in '*Aerobacter aerogenes* or *Klebsiella*' by Gibson & Magrath (1969). Similar findings were reported by Braun (1981). Valvano & Crosa (1984) found that the gene determining aerobactin-production can be chromosomal in K1 antigen-carrying strains. The ability to multiply in blood and tissue is dependent on the availability of iron for the support of bacterial growth. Aerobactin and other iron-binding compounds in association with haemolysis could therefore be looked upon as highly important synergistic virulence factors.

Adhesins

Presence of adhesins in strains from sepsis and meningitis has not been examined as extensively as it has in those from UTI. It is known that O6:K2:H1 and O4:K12:H5 strains from blood have the same fimbrial antigens as the UTI strains of these serotypes (Ørskov & Ørskov, 1983); however, Korhonen *et al.* (1985) have reported that O6:K2:H1 and O7:K1:H- strains from neonatal sepsis or meningitis apart from the gal-gal binding fimbriae also produced X adhesins, i.e. adhesins not identified as type 1, gal-gal (P), S or M fimbriae, and furthermore that most of the O18:K1:H7 strains from such cases had no gal-gal fimbriae but S fimbriae, i.e. those recognizing sialyl galactosides. For further details regarding adhesins see under UTI strains.

CONCLUSION

The *Escherichia coli* species consists of a bewildering number of stable phenotypes. The highly selected strains received in Copenhagen since 1950 (more than 30000 strains) belong to several thousand different serotypes, not to speak of the much

higher number of bio-serotypes or more highly defined phenotypes into which they could be subdivided.

It is therefore consoling for the medically orientated person that recent times have seen a revival of the ideas which were put forward many years ago (Sjöstedt, 1946), i.e. that each well-defined O:K:H serotype had a characteristic pathogenic capacity, and that the number of virulent serotypes was quite restricted. The possibility that such well-defined O:K:H serotypes might represent bacterial clones was suggested by Ørskov *et al.* (1976), based on investigations on enterotoxigenic *E. coli* (ETEC) isolated all over the world. This clone concept was also proposed as an explanation for the non-random occurrence of serobiotypes O18ac:K1:H7, O1:K1:H7 and O16:K1:H6 in strains from blood and faeces (Myerowitz *et al.* 1977). Achtman *et al.* (1983) introduced the analysis of outer membrane proteins (OMP) and Caugant *et al.* (1981) the iso-enzyme analysis, both important for a detailed clone definition.

The pathogenic phenotypes are characterized by certain virulence factors; however, a single trait is not enough to cause virulence, several traits must act in concert.

Recent investigations also indicate that not only do some special clones, that often can be described by the sero-biotype, have a special virulence, but it is also apparent that disease like neonatal meningitis, non-neonatal bacteraemia and pyelonephritis, i.e. diseases with different locations in the human body, are sometimes connected with special types characteristic for each disease location.

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REFERENCES

Achtman, M., Mercer, A., Kusecek, B., Pohl, A., Heuzenroeder, M., Aronson, W., Sutton, A. & Silver, R. P. (1983). Six widespread clones among *Escherichia coli* K 1 isolates. *Infection and Immunity* **39**, 315–335.

Bar-Sharit, Z., Ofek, I., Goldman, D., Mirelman, D. & Sharon, N. (1977). Mannose residues on phagocytes as receptors for the attachment of *Escherichia coli* and *Salmonella typhi*. *Biomedical and Biophysical Research Communication* **78**, 455–460.

Barry, G. T. & Goebel, W. F. (1957). Colominic acid, a substance of bacterial origin related to sialic acid. *Nature* (*London*) **179**, 206–208.

Bergström, T., Lincoln, K., Orskov, F., Orskov, I. & Winberg, J. (1967). Studies of urinary tract infections in infancy and childhood. *Journal of Pediatrics* **71**, 13–20.

Bettelheim, K. A. & Taylor, J. (1969). A study of *Escherichia coli* isolated from chronic urinary infections. *Journal of Medical Microbiology* **2**, 225–236.

Björkstén, B., Bortolussi, R., Gothefors, L. & Quie, P. G. (1976). Interaction of *E. coli* strains with human serum: Lack of relationship to K1 antigen. *Journal of Pediatrics* **89**, 892–899.

Björkstén, B. & Kaijser, B. (1978). Interaction of human serum and neutrophils with *Escherichia coli* strains: difference between strains isolated from urine of patients with pyelonephritis or asymptomatic bacteriuria. *Infection and Immunity* **22**, 308–311.

Blumenstock, E. & Jann, K. (1982). Adhesion of piliated *Eschericia coli* strains to phagocytes: difference between bacteria with mannose-sensitive pili and those with mannose-resistant pili. *Infection and Immunity* **35**, 264–269.

Bolanos, R. & de Witt, C. W. (1966). Isolation and characterization of the K1 (L) antigen of Escherichia coli. *Journal of Bacteriology* **91**, 987–996.

BOLLGREN, I. & WINBERG, J. (1976). The periurethral aerobic bacterial flora in girls highly susceptible to urinary infections. *Acta Paediatrica Scandinavica* **65**, 81–87.

BORTOLUSSI, R., FERRIERI, P., BJÖRKSTÉN, B. & QUIE, P. B. (1979). Capsular K 1 polysaccharide of *Escherichia coli*. Relationship to virulence in newborn rats and resistance to phagocytosis. *Infection and Immunity* **25**, 293–298.

BRAUN, V. (1981). *Escherichia coli* cells containing the plasmid Col V produce the iron ionophore aerobactin. *FEMS Microbiology Letters* **11**, 225–228.

BRINTON, C. C., JR, BUZZELL, A. & LAUFFER, M. A. (1954). Electrophoresis and phage susceptibility studies on a filament-producing variant of the *E. coli* B bacterium. *Biochemical and Biophysical Acta* **15**, 533–542.

BROOKS, H. J. L., O'GRADY, F., MCSHERRY, A. & CATTELL, W. R. (1980). Uropathogenic properties of *Escherichia coli* in recurrent urinary tract infection. *Journal of Medical Microbiology* **13**, 57–68.

CAUGANT, D. A., LEVIN, B. R. & SELANDER, R. K. (1981). Genetic diversity and temporal variation in the *E. coli* population of a human host. *Genetics* **98**, 467–490.

CAVALIERI, S. J. & SNYDER, I. S. (1982*a*). Cytotoxic activity of partially purified *Escherichia coli* alpha haemolysin. *Journal of Medical Microbiology* **15**, 11–21.

CAVALIERI, S. J. & SNYDER, I. S. (1982*b*). Effect of *Escherichia coli* alpha-hemolysin on human peripheral leucocyte viability in vitro. *Infection and Immunity* **36**, 455–461.

CAVALIERI, S. J. & SNYDER, I. S. (1982*c*). Effect of *Escherichia coli* alpha-hemolysin on human peripheral leucocyte function in vitro. *Infection and Immunity* **37**, 966–974.

CHEASTY, T., GROSS, R. J. & ROWE, B. (1977). Incidence of K 1 antigen in *Escherichia coli* isolated from blood and cerebrospinal fluid of patients in the United Kingdom. *Journal of Clinical Pathology* **30**, 945–950.

CHICK, S., HARBER, M. J., MACKENZIE, R. & ASSCHER, A. W. (1981). Modified method for studying bacterial adhesion to isolated uroepithelial cells and uromucoid. *Infection and Immunity* **34**, 256–261.

COOKE, E. M. & EWINS, S. P. (1975). Properties of strains of *Escherichia coli* isolated from a variety of sources. *Journal of Medical Microbiology* **8**, 107–111.

CROSS, A. S., GEMSKI, P., SADOFF, J. C., ØRSKOV, F. & ØRSKOV, I. (1984). The importance of the K 1 capsule in invasive infections caused by *Escherichia coli*. *Journal of Infectious Diseases* **149**, 184–193.

DE BOY, J. M., WACHSMUTH, J. K. & DAVIS, B. R. (1980). Hemolytic activity in enterotoxigenic and non enterotoxigenic strains of *Escherichia coli*. *Journal of Clinical Microbiology* **12**, 193–198.

DUDGEON, L. S., WORDLEY, E. & BAWTREE, F. (1921). On *Bacillus coli* infections of the urinary tract, especially in relation to haemolytic organisms. *Journal of Hygiene* A **20**, 137–164.

DUGUID, J. P. & GILLIES, R. R. (1957). Fimbriae and adhesive properties of dysentery bacilli. *Journal of Pathology and Bacteriology* **74**, 397–411.

DUGUID, J. P. & OLD, D. C. (1980). Adhesive properties of Enterobacteriaceae. In *Receptors and Recognition* (ed. E. H. Beachy), series B, vol. 6, pp. 185–217. London: Chapman & Hall.

EMÖDY, L., PAL, N., SAFONOVA, N. V., KUCH, B. & GOLUTVA, N. K. (1980). Alpha-haemolysin: an additive virulence factor in *Escherichia coli*. *Acta Microbiologica Academiae Scientiarum Hungaricae* **27**, 333–342.

ESCHERICH, T. (1885*a*). Die Darmbakterien des Neugeborenen und Säuglings. *Fortschritte der Medizin* **3**, 515–522.

ESCHERICH, T. (1885*b*). Die Darmbakterien des Neugeborenen und Säuglings. *Fortschritte der Medizin* **3**, 547–554.

EVANS, D. J., EVANS, D. G., HÖHNE, C., NOBLE, M. A., HALDANE, E. V., LIOR, H. & YOUNG, L. S. (1981). Hemolysin and K antigens in relation to serotype and hemagglutination type of *Escherichia coli* isolated from extraintestinal infection. *Journal of Clinical Microbiology* **13**, 171–178.

EWERTSEN, H. W. (1946). *Dyreexperimentelle Undersøgelser over Colibacillernes Patogenitet og Effekten af Coliserum*, pp. 1–192. Copenhagen: Arnold Busck.

FOWLER, J. E. JR & STAMEY, T. A. (1977). Studies of introital colonization in women with recurrent urinary infections. VII. The role of bacterial adherence. *Journal of Urology* **117**, 472–476.

GADEBERG, O. V. & ØRSKOV, I. (1984). In vitro cytotoxic effect of α-hemolytic *Escherichia coli* on human blood granulocytes. *Infection and Immunity* **45**, 255–260.

GADEBERG, O. V., ØRSKOV, I. & RHODES, J. M. (1983). Cytotoxic effect of an alpha-hemolytic *Escherichia coli* strain on human blood monocytes and granulocytes in vitro. *Infection and Immunity* **41**, 358–364.

GIBSON, F. & MAGRATH, D. I. (1969). The isolation and characterization of a hydroxamic acid (aerobactin) formed by *Aerobacter aerogenes* 62-1. *Biochemical and Biophysical Acta* **192**, 175–184.

GLYNN, A. A., BRUMFITT, W. & HOWARD, C. J. (1971). K antigens of *Escherichia coli* and renal involvment in urinary tract infections. *Lancet* i, 514–516.

GLYNN, A. A. & HOWARD, C. J. (1970). The sensitivity to complement of strains of *Escherichia coli* related to their K antigens. *Immunology* **18**, 331–346.

GOLDHAR, J., PERRY, R. & OFEK, I. (1984). Extraction and properties of nonfimbrial mannose-resistant hemagglutinin from a urinary isolate of *Escherichia coli*. *Current Microbiology* **11**, 49–54.

GOLDMAN, R. C., JOINER, K. & LEIVE, L. (1984). Serum resistant mutants of *Escherichia coli* O111 contain increased lipopolysaccharide, lack an O antigen containing capsule and have more of their lipid A core with O antigen. *Journal of Bacteriology* **159**, 877–882.

GOWER, P. E., TAYLOR, P. W., KOUTSAIMANIS, K. G. & ROBERTS, A. P. (1972). Serum bactericidal activity in patients with upper and lower urinary tract infections. *Clinical Science and Molecular Medicine* **43**, 13–22.

GRADOS, O. & EWING, W. H. (1970). Antigenic relationship between *Escherichia coli* and *Neisseria meningitidis*. *Journal of Infectious Diseases* **122**, 100–103.

GROSS, R. J., CHEASTY, T. & ROWE, B. (1977). Isolation of bacteriophages specific for the K1 polysaccharide antigen of *Escherichia coli*. *Journal of Clinical Microbiology* **6**, 548–550.

GROSSMAN, N. & LEIVE, L. (1984). Complement activation via the alternative pathway by purified Salmonella lipopolysaccharide is affected by its structure but not its O antigen length. *Journal of Immunology* **132**, 376–385.

GRÜNEBERG, R. N. (1969). Relationship of infecting urinary organisms to the fecal flora in patients with symptomatic urinary infection. *Lancet* ii, 766–768.

GRÜNEBERG, R. N., LEIGH, D. A. & BRUMFITT, W. (1968). *Escherichia coli* serotypes in urinary infection: studies in domiciliary, antenatal and hospital practice. In *Urinary Tract Infection* (ed. F. O'Grady & W. Brumfitt), pp. 68–79. London: Oxford University Press.

HACKER, J., SCHRÖTER, G., SCHRETTENBRUNNER, A., HUGHES, C. & GOEBEL, W. (1983). Hemolytic *Escherichia coli* strains in the human fecal flora as potential urinary pathogens. *Zentralblatt für Bakteriologie und Hygiene* (I. Abt. Orig. A) **254**, 370–378.

HAGBERG, L., ENGBERG, I., FRETER, R., LAM, J., OLLING, S. & SVANBORG EDÉN, C. (1983*a*). Ascending, unobstructed urinary tract infection in mice caused by pyelonephritogenic *Escherichia coli* of human origin. *Infection and Immunity* **40**, 273–283.

HAGBERG, L., HULL, R., HULL, S., FALKOW, S., FRETER, R. & SVANBORG EDÉN, C. (1983*b*). Contribution of adhesin to bacterial persistence in the mouse urinary tract. *Infection and Immunity* **40**, 265–272.

HAGBERG, L., HULL, R., HULL, S., MCGHEE, J. R., MICHALEK, S. M. & SVANBORG EDÉN, C. (1984). Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. *Infection and Immunity* **46**, 839–844.

HAGBERG, L., JODAL, U., KORHONEN, T. K., LIDIN-JANSON, G., LINDBERG, U. & SVANBORG EDÉN, C. (1981). Adhesion, haemagglutination, and virulence of *Escherichia coli* causing urinary tract infections. *Infection and Immunity* **31**, 564–570.

HANDRÜCK, W., STEINRÜCK, H., SPENCKER, F.-B., BRAUN, W. & VOGTMAN, C. (1982). Infektionen durch *E. coli* in der Neonatalperiode. II. Bericht über 61 Erkrankungen. *Deutsches Gesundheitswesen* **37**, 2212–2219.

HANSON, L. Å., AHLSTEDT, S., FASTH, A., JODAL, U., KAIJSER, B., LARSSON, P., LINDBERG, U., OLLING, S., SOHL-ÅKERLUND, A. & SVANBORG-EDÉN, C. (1977). Antigens of *Escherichia coli*, human immune response, and the pathogenesis of urinary tract infections. *Journal of Infectious Diseases* **136**, Suppl. S144–149.

HARBER, M. J., CHICK, S., MACKENZIE, R. & ASSCHER, A. W. (1982). Lack of adherence to epithelial cells by freshly isolated urinary pathogens. *Lancet* i, 586–588.

HENKEL, W. (1970). Serumresistenz und Nephropathogenität antigenetisch definierten Stämme von *E. coli*. *Zeitschrift für Medizinische Mikrobiologie und Immunologie* **156**, 44–59.

HUGHES, C., PHILLIPS, R. & 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. *Infection and Immunity* **35**, 270–275.

HULL, R. A., GILL, R. E., HSU, P., MINSHEW, B. H. & FALKOW, S. (1981). Construction and expression of recombinant plasmids encoding type 1, or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. *Infection and Immunity* **33**, 933–938.

JANN, K. & JANN, B. (1983). The K antigens of *Escherichia coli*. *Progress in Allergy* **33**, 53–79.

JANN, K., JANN, B. & SCHMIDT, G. (1981). SDS polyacrylamide gel electrophoresis and serological analysis of pili from *Escherichia coli* of different pathogenic origin. *FEMS Microbiology Letters* **11**, 21–25.

KAIJSER, B. (1973). Immunology of *Escherichia coli*: K antigen and its relation to urinary-tract infection. *Journal of Infectious Diseases* **127**, 670–677.

KAIJSER, B., HANSON, L. Å., JODAL, U., LIDIN-JANSON, G. & ROBBINS, J. B. (1977). Frequency of *E. coli* K antigens in urinary-tract infections in children. *Lancet* i, 663–664.

KÄLLENIUS, G. & MÖLLBY, R. (1979). Adhesion of *Escherichia coli* to human periurethral cells correlated to mannose-resistant agglutination of human erythrocytes. *FEMS Microbiology Letters* **5**, 295–299.

KÄLLENIUS, G., MÖLLBY, R. & WINBERG, J. (1980). In vitro adhesion of uropathogenic *Escherichia coli* to human periurethral cells. *Infection and Immunity* **28**, 972–980, 1980.

KÄLLENIUS, G., MÖLLBY, R., SVENSON, S. B., WINBERG, J. & HULTBERG, H. (1980). Identification of a carbohydrate receptor recognized by uropathogenic *Escherichia coli*. *Infection*, Suppl. 3, S288–293.

KÄLLENIUS, G., SVENSON, S. B., HULTBERG, H., MÖLLBY, R., HELIN, I., CEDERGREN, B. & WINBERG, J. (1981). Occurrence of P-fimbriated *Escherichia coli* in urinary tract infections. *Lancet* ii, 1369–1372.

KAUFFMANN, F. (1940). Zur Serologie des I-Antigens in der Salmonella Gruppe. *Acta Pathologica et Microbiologica Scandinavica* **17**, 135–144.

KAUFFMANN, F. (1943). Über neue thermolabile Körperantigene der Coli-Bakterien. *Acta Pathologica et Microbiologica Scandinavica* **20**, 21–44.

KAUFFMANN, F. (1944*a*). Zur Serologie der Coli-Gruppe. *Acta Pathologica et Microbiologica Scandinavica* **21**, 20–45.

KAUFFMANN, F. (1944*b*). Untersuchungen über die Körper-Antigene der Coli-Bakterien. *Acta Pathologica et Microbiologica Scandinavica* **21**, 46–64.

KAUFFMANN, F. (1947). The serology of the Coli group. *Journal of Immunology* **57**, 71–100.

KAUFFMANN, F. & VAHLNE, G. (1945). Über die Bedeutung des serologischen Formenwechsels für de Bakteriophagenwirkung in der Coli-Gruppe. *Acta Pathologica et Microbiologica Scandinavica* **22**, 119–137.

KÉTYI, I. (1981). Suckling mouse model of urinary tract infections caused by *Escherichia coli*. *Acta Microbiologica Academiae Scientiarum Hungaricae* **28**, 393–399.

KÉTYI, I., EMÖDY, L., KONTROHR, T., VERTENYI, A., PACSA, S., AVDEEVA, T. A., SAFONOVA, N. V. & GOLUTOVA, N. K. (1978). Mouse lung oedema caused by a toxic substance of *Escherichia coli* strains. *Acta Microbiologica Academiae Scientiarum Hungaricae* **25**, 307–317.

KÉTYI, I., NAUMANN, G. & NIMMICH, W. (1983). Urinary tract infectivity of R strains of *Escherichia coli* carrying various virulence factors. *Acta Microbiologica Academiae Scientiarum Hungaricae* **30**, 155–161.

KLEMM, P., ØRSKOV, I., & ØRSKOV, F. (1983). Isolation and characterization of F12 adhesive fimbrial antigen from uropathogenic *Escherichia coli* strains. *Infection and Immunity* **40**, 91–96.

KNIPSCHILDT, H. E. (1945). *Undersogelser over Coligruppens Serologi*, pp. 1–156. Copenhagen: Nyt Nordisk Forlag, Arnold Busck.

KORHONEN, T. K., LEFFLER, H. & SVANBORG EDÉN, C. (1981). Binding specificity of piliated strains of *Escherichia coli* and *Salmonella typhimurium* to epithelial cells, *Saccharomyces cerevisiae* and erythrocytes. *Infection and Immunity* **32**, 796–804.

KORHONEN, T. K., VÄISÄNEN, V., SAXÉN, H., HULTBERG, H. & SVENSON, S. B. (1982). P-antigen-recognizing fimbriae from human uropathogenic *Escherichia coli* strains. *Infection and Immunity* **37**, 286–291.

KORHONEN, T. K., VÄISÄNEN-RHEN, V., RHEN, M., PERE, A., PARKKINEN, J. & FINNE, J. (1984). *Escherichia coli* fimbriae recognizing sialyl galactosides. *Journal of Bacteriology* **159**, 762–766, 1984.

KORHONEN, T. K., VALTONEN, M. V., PARKKINEN, J., VÄISÄNEN-RHEN, V., FINNE, J., ORSKOV, F., ORSKOV, I., SVENSON, S. B. & MÄKELÄ, P. H. (1985) *Escherichia coli* strains associated with neonatal sepsis and meningitis: Serotypes, hemolysin production and receptor recognition. *Infection and Immunity* **48**, 486–491.

KREGER, B. E., CRAVEN, D. E., CARLING, P. C. & MCCABE, W. R. (1980). Gram-negative bacteremia. III. Reassessment of etiology, epidemiology and ecology in 612 patients. *American Journal of Medicine* **68**, 332–343.

KUNIN, C. M. (1982). Urinary tract infection: new information concerning pathogenesis and management. *Journal of Urology* **128**, 1233.

KUNIN, C. M., DEUTSCHER, R. & PAQUIN, A. (1964). Urinary tract infection in school children: an epidemiological, clinical and laboratory study. *Medicine* **43**, 91–130.

LABIGNE-ROUSSEL, A. F., LARK, D., SCHOOLNIK, G. & FALKOW, S. (1984). Cloning and expression of an afimbrial adhesin (AFA-I) responsible for P blood group-independent, mannose-resistant hemagglutination from a pyelonephritic *Escherichia coli* strain. *Infection and Immunity* **46**, 251–259.

LEFFLER, H. & SVANBORG EDÉN, C. (1981). Chemical identification of a glycosphincolipid receptor for *Escherichia coli* attaching to human urinary epithelial cells and agglutinating human erythrocytes. *FEMS Microbiology Letters* **8**, 127–134.

LIDIN-JANSON, G., HANSON, L. Å., KAIJSER, B., LINCOLN, K., LINDBERG, U., OLLING, S. & WEDEL, H. (1977). Comparison of *Escherichia coli* from bacteriuric patients with those from faeces of healthy school children. *Journal of Infectious Diseases* **136**, 346–353.

LINDBERG, U., HANSON, L. Å., JODAL, U., LIDIN-JANSON, G., LINCOLN, K. & OLLING, S. (1975*a*). Asymptomatic bacteriuria in schoolgirls. II. Differences in *Escherichia coli* causing asymptomatic and symptomatic bacteriuria. *Acta Paediatrica Scandinavica* **64**, 432–436.

LINDBERG, U., JODAL, U., HANSON, L. Å. & KAIJSER, B. (1975*b*). Asymptomatic bacteriuria in schoolgirls. IV. Difficulties of level diagnosis and the possible relation to the character of infecting bacteria. *Acta Paediatrica Scandinavica* **64**, 574–580.

LINGGOOD, M. A. & INGRAM, P. L. (1982). The role of alpha haemolysin in the virulence of *Escherichia coli* for mice. *Journal of Medical Microbiology* **15**, 23–30.

LOMBERG, H., HELLSTRÖM, M., JODAL, U., LEFFLER, H., LINCOLN, K. & SVANBORG EDÉN, C. (1984). Virulence-associated traits in *Escherichia coli* causing first and recurrent episodes of urinary tract infection in children with or without reflux. *Journal of Infectious Diseases* **150**, 561–569.

MABECK, C. E., ØRSKOV, F. & ØRSKOV, I. (1971*a*). *Escherichia coli* serotypes and renal involvement in urinary tract infection. *Lancet* i, 1312–1314.

MABECK, C. E., ØRSKOV, F. & ØRSKOV, I. (1971*b*). Studies in urinary tract infections. VIII. *Escherichia coli* O:H serotypes in recurrent infections. *Acta Medica Scandinavica* **190**, 279–282.

MCCABE, W. R., CARLING, P. C., BRUINS, S. & GREELY, A. (1975). The relation of K antigen to virulence of *Escherichia coli*. *Journal of Infectious Diseases* **131**, 6–10.

MCCABE, W. R., KAIJSER, B., OLLINGS, J., UWAYDAH, M. & HANSON, L. Å. (1978). *Escherichia coli* in bacteremia: K and O antigens and serum sensitivity of strains from adults and neonates. *Journal of Infectious Diseases* **138**, 33–41.

MCCRACKEN, G. H., SARFF, L. D., GLODE, M. P., MIZE, S. G., SCHIFFER, M. S., ROBBINS, J. B., GOTSCHLICH, E. C. ØRSKOV, I. & ØRSKOV, F. (1974). Relation between *Escherichia coli* K1 capsular polysaccharide antigen and clinical outcome in neonatal meningitis. *Lancet* ii, 246–250.

MCGEACHIE, J. (1965). Serological grouping of urinary *Escherichia coli*. *Journal of Clinical Pathology* **18**, 428–431.

MAIZTEGUI, J. I., BIEGELEISEN, J. Z. JR, CHERRY, W. B. & KAAS, E. H. (1965). Bacteriemia due to gram-negative rods. A clinical bacteriologic, serologic, and immunofluorescent study. *New England Journal of Medicine* **272**, 222–229.

MANGAN, D. F. & SNYDER, I. S. (1979). Mannose-sensitive interaction of *Escherichia coli* with human peripheral leucocytes in vitro. *Infection and Immunity* **26**, 520–527.

MINSHEW, B., JORGENSEN, J., COUNTS, G. W. & FALKOW, S. (1978). Association of hemolysin production, hemagglutination of human erythrocytes, and virulence for chicken embryos of extraintestinal *Escherichia coli* isolates. *Infection and Immunity* **20**, 50–54.

MOLL, A., MANNING, P. A. & TIMMIS, K. N. (1980). Plasmid-determined resistance to serum activity: a major membrane protein, the *tra T* gene product is responsible for plasmid specified serum resistance in *Escherichia coli*. *Infection and Immunity* **28**, 359–367.

MULDER, C. J. J., VAN ALPHEN, L. & ZANEN, H. C. (1984). Neonatal meningitis caused by *Escherichia coli* in The Netherlands. *Journal of Infectious Diseases* **150**, 935–940.

MULDER, C. J. J. & ZANEN, H. C. (1984). A study of 280 cases of neonatal meningitis in The Netherlands. *Journal of Infection* **8**, 177–184.

MYEROWITZ, R. L., ALBERS, A. C., YEE, R. B. & ØRSKOV, F. (1977). Relationship of K 1 antigen to biotype in clinical isolates of *Escherichia coli*. *Journal of Clinical Microbiology* **6**, 124–127.

NOWICKI, B., RHEN, M., VÄISÄNEN-RHEN, V., PERE, A. & KORHONEN, T. K. (1984). Immunofluorescence study of fimbrial phase variation in *Escherichia coli* KS71. *Journal of Bacteriology* **160**, 691–695.

O'HANLEY, P., LARK, D., FALKOW, S. & SCHOOLNIK, G. (1985). Molecular basis of *Escherichia coli* colonization of the upper urinary tract in BALB/c mice: gal-gal pili immunization prevents *E. coli* pyelonephritis. *Journal of Clinical Investigation* **75**, 347–360.

OLLING, S. (1977). Sensitivity of gram-negative bacilli to the serum bactericidal activity: A marker of host-parasite relationship in acute and persisting infections. *Scandinavian Journal of Infectious Diseases*, Suppl. 10, 1–40.

OLLING, S., HANSON, L. A., HOLMGREN, J., JODAL, U., LINCOLN, K. & LINDBERG, U. (1973). The bactericidal effect of normal human serum on *E. coli* strains from normals and from patients with urinary tract infections. *Infection* **1**, 24–28.

OPAL, S., CROSS, A. & GEMSKI, P. (1982). K antigen and serum sensitivity of rough *Escherichia coli*. *Infection and Immunity* **37**, 956–960.

ØRSKOV, I., BIRCH-ANDERSEN, A., DUGUID, J., STENDERUP, J. & ØRSKOV, F. (1985). An adhesive protein capsule of *Escherichia coli*. *Infection and Immunity* **47**, 191–200.

ØRSKOV, I., FERENCZ, A., & ØRSKOV, F. (1980). Tamm–Horsfall protein or uromucoid is the normal urinary slime that traps type 1 fimbriated *Escherichia coli*. *Lancet* i, 887.

ØRSKOV, F. & ØRSKOV, I. (1967). In: Round table conference on non-obstructive urinary tract infections in children. Proceedings of the 15th Northern Pediatric Congress, Bergen, 1967. *Acta Paediatrica Scandinavica*, suppl. **177**, 44–45.

ØRSKOV, F. & ØRSKOV, I. (1975). *Escherichia coli* O:H serotypes isolated from human blood. *Acta Pathologica et Microbiologica Scandinavica* **B 83**, 585–600.

ØRSKOV, I. & ØRSKOV, F. (1983). Serology of *Escherichia coli* fimbriae. *Progress in Allergy* **33**, 80–105.

ØRSKOV, I. & ØRSKOV, F. (1985). Some *Escherichia coli* adhesins. In *Seminars in Infectious Diseases*. Vol. V. *Bacterial Vaccines*. (In the Press.)

ØRSKOV, I., ØRSKOV, F. & BIRCH-ANDERSEN, A. (1980). Comparison of *Escherichia coli* fimbrial antigen F7 with type 1 fimbriae. *Infection and Immunity* **27**, 657–666.

ØRSKOV, I., ØRSKOV, F., BIRCH-ANDERSEN, A., KANAMORI, M. & SVANBORG EDÉN, C. (1982*b*). O, K, H and fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. *Scandinavian Journal of Infectious Diseases*, suppl. **33**, 18–25.

ØRSKOV, I., ØRSKOV, F., BIRCH-ANDERSEN, A., KLEMM, P. & SVANBORG EDÉN, C. (1982*a*). Protein attachment factors: fimbriae in adhering *Escherichia coli*. In *Seminars in Infectious Diseases*. Vol. IV. *Bacterial Vaccines*, pp. 97–103. New York: Thieme-Stratton.

ØRSKOV, F., ØRSKOV, I., EVANS, D. J., SACK, R. B., SACK, P. A. & WADSTRÖM, T. (1976). Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. *Medical Microbiology and Immunology* **162**, 73–80.

ØRSKOV, F., ØRSKOV, I., JANN, B. & JANN, K. (1971). Immunoelectrophoretic patterns of extracts from all *Escherichia coli* O and K antigen test strains. Correlation with pathogenicity. *Acta Pathologica et Microbiologica Scandinavica*, **B**, **79**, 142–152.

ØRSKOV, I., ØRSKOV, F., JANN, B. & JANN, K. (1977). Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. *Bacteriological Reviews* **41**, 667–710.

ØRSKOV, F., ØRSKOV, I., SUTTON, A., SCHNEERSON, R., LIN, W., EGEN, W., HOFF, G. E. & ROBBINS, J. B. (1979). Form variation in *Escherichia coli* K1: Determined by O-acetylation of the capsular polysaccharide. *Journal of Experimental Medicine* **149**, 669–685.

PARKKINEN, J., FINNE, J., ACHTMAN, M., VÄISÄNEN, V. & KORHONEN, T. K. (1983). *Escherichia coli* strains binding neuraminyl α2-3 galactosides. *Biochemical and Biophysical Research Communications* **111**, 456–461.

PLUSCHKE, G. & ACHTMAN, M. (1984). Degree of antibody-independent activation of the classical complement pathway by K1 *Escherichia coli* differs with O antigen type and correlates with virulence of meningitis in newborns. *Infection and Immunity* **43**, 684–692.

PLUSCHKE, G., MAYDEN, J., ACHTMAN, M. & LEVINE, R. P. (1983). Role of capsule and the O antigen in resistance of O18:K1 *Escherichia coli* to complement-mediated killing. *Infection and Immunity* **42**, 907–913.

QUACKENBUSH, R. L. & FALKOW, S. (1979). Relationship between Colicin V activity and virulence in *Escherichia coli*. *Infection and Immunity* **24**, 562–564.

RANTZ, L. A. (1962). Serological grouping of *Escherichia coli*. *Archives of Internal Medicine* **109**, 37–42.

RHEN, M., MÄKELÄ, P. H. & KORHONEN, T. K. (1983). P-fimbriae of *Escherichia coli* are subject to phase variation. *FEMS Microbiology Letters* **19**, 267–271.

ROBBINS, J. B., MC CRACKEN, G. H., GOTSCHLICH, E. C., ORSKOV, F., ORSKOV, I. & HANSON, L. Å. (1974*a*). *Escherichia coli* K 1 capsular polysaccharide associated with neonatal meningitis. *New England Journal of Medicine* **290**, 1216–1220.

ROBBINS, J. B., PARKE, J. C., JR, SCHNEERSON, R. & WHISNANT, J. K. (1973). Quantitative measurement of 'natural' and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. *Pediatric Research* **7**, 103–110.

ROBBINS, J. B., SCHNEERSON, R., LIU, T. Y., SCHIFFER, M. S., SCHIFFMAN, G., MYEROWITZ, R. L., MCCRACKEN, G. H., ØRSKOV, I. & ØRSKOV, F. (1974*b*). Cross-reacting bacterial antigens and immunity to disease caused by encapsulated bacteria. *4th International Convocation on Immunology, Buffalo, N.Y.*, 1974 (ed. E. Neter and F. Milgrom), pp. 218–241. Basel: S. Karger.

ROBBINS, J. B., SCHNEERSON, R., PARKE, J. C., LIU, T. Y., HANDZEL, Z. T., ORSKOV, I. & ØRSKOV, F. (1976). *Escherichia coli* O 75 : Kf 147 : H 5 cross reactive with capsular polysaccharide of *Haemophilus influenzae* type B: studies of the immunochemical relationship and properties of heteroimmunization in humans to prevent septicemic disease caused by *H. influenzae* type B. In *The Role of Immunological Factors in Infections, Allergy and Autoimmune Processes* (ed. R. F. Beers Jr and E. G. Bassett), pp. 103–120. New York: Raven Press.

ROWLEY, D. (1968). Sensitivity of rough gram-negative bacteria to the bactericidal action of serum. *Journal of Bacteriology* **95**, 1647–1650.

SACK, R. & MILLER, C. E. (1969). Progressive changes of Vibrio serotypes in germ-free mice infected with *Vibrio cholerae*. *Journal of Bacteriology* **99**, 688–695.

SALIT, I. E. & GOTSCHLICH, E. C. (1977). Type 1 *Escherichia coli* pili: characterization of binding to monkey kidney cells. *Journal of Experimental Medicine* **146**: 1182–1194.

SARFF, L. D., MCCRACKEN, G. H., SCHIFFER, M. S., GLODE, M. P., ROBBINS, J. B., ORSKOV, I. & ØRSKOV, F. (1975). Epidemiology of Escherichia coli K 1 in healthy and diseased newborns. *Lancet* i, 1099–1104.

SCHAEFFER, A. J., AMUNDSEN, S. K. & SCHMIDT, L. N. (1979). Adherence of *Escherichia coli* to human urinary tract epithelial cells. *Infection and Immunity* **24**: 753–759.

SCHAEFFER, A. J., JONES, J. M., DUNCAN, J. L., CHMIEL, J. S., PLOTKIN, B. J. & FALKOWSKI, W. S. (1982). Adhesion of uropathogenic *Escherichia coli* to epithelial cells from women with recurrent urinary tract infection. *Infection* **10**, 186–191.

SCHNEERSON, R., BRADSHAW, M., WHINANT, J. K., MYEROWITZ, R. L., PARKE, J. C., JR & ROBBINS, J. B. (1972). An *Escherichia coli* antigen cross-reactive with capsular polysaccharide of *Haemophilus influenzae* type b. *Journal of Immunology* **108**, 1551–1562.

SIETZEN, W. (1979). Typisierung von *Escherichia-coli*-O- und -K-Antigenen bei Patienten mit Harnwegsinfektionen. *Infektion* **7**, Suppl. 2, S 197–S 206.

SILVER, R. P., FINN, C. W., VANN, W. F., ARONSON, W., SCHNEERSON, R., KRETSCHMER, P. J. & GARON, C. F. (1981). Molecular cloning of the K 1 capsular polysaccharide genes of *E. coli*. *Nature* (*London*) **289**, 696–698.

SILVER, R. P., VANN, W. F. & ARONSON, W. (1984). Genetic and molecular analysis of *Escherichia coli* K 1 antigen genes. *Journal of Bacteriology* **157**, 568–575.

SILVERBLATT, F. J., DREYER, J. S. & SCHAUER, S. (1979). Effect of pili on susceptibility of *Escherichia coli* to phagocytosis. *Infection and Immunity* **24**, 218–223.

SJÖSTEDT, S. (1946). Pathogenicity of certain serological types of *E. coli*. Their mouse toxicity, hemolytic power, capacity for skin necrosis and resistance to phagocytosis and bactericidal faculties of human blood. *Acta Pathologica et Microbiologica Scandinavica*, Suppl. **63**, 1–148.

SMITH, H. W. (1963). The haemolysins of *Escherichia coli*. *Journal of Pathology and Bacteriology* **83**, 197–211.

SMITH, H. W. & HUGGINS, M. B. (1980). The association of O 18, K 1 and H 7 antigens and the Col V plasmid of a strain of *Escherichia coli* with its virulence and immunogenicity. *Journal of General Microbiology* **121**, 387–400.

SMITH, H. W. & HUGGINS, M. B. (1985). The toxic role of alphahaemolysin in the pathogenesis of experimental *Escherichia coli* infection in mice. *Journal of General Microbiology* **131**, 395–403.

SMITH, H. W. & LINGGOOD, M. A. (1971). Observations on the pathogenic properties of the K 88, Hly and Ent plasmids of *Escherichia coli* with particular reference to porcine diarrhoea. *Journal of Medical Microbiology* **4**, 467–485.

SMITH, T. (1927). Studies on pathogenic *B. coli* from bovine sources. III. Normal and serological induced resistance to *B. coli* and its mutant. *Journal of Experimental Medicine* **46**, 141–153.

STAMM, W. E., COUNTS, G. W., RUNNING, K. R., FILM, S., TURCK, M. & HOLMES, K. K. (1982). Diagnosis of coliform infection in acutely dysuric women. *New England Journal of Medicine* **307**, 463–468.

STEVENS, P., CHU, C. L. & YOUNG, L. S. (1980). K1 antigen content and the presence of an additional sialic acid containing antigen among bacteriemic K1 *Escherichia coli*: correlation with susceptibility to opsonophagocytosis. *Infection and Immunity* **29**, 1055–1061.

STEVENS, P., HUANG, N.-Y., WELLS, W. D. & YOUNG, L. S. (1978). Restricted complement activation by *Escherichia coli* with K1 capsular serotype: a possible role in pathogenicity. *Journal of Immunology* **121**, 2174–2180.

SVANBORG EDÉN, C., BJURSTEN, L. M., HULL, R., HULL, S., MAGNUSSON K.-E., MOLDOVANO, Z. & LEFFLER, H. (1984). Influence of adhesins on the interaction of *Escherichia coli* with human phagocytes. *Infection and Immunity* **44**, 672–680.

SVANBORG EDÉN, C., ERIKSSON, B., HANSON, L. Å., JODAL, U., KAIJSER, B., LIDIN JANSON, G., LINDBERG, U. & OLLING, S. (1978). Adhesion to normal human uroepithelial cells of *Escherichia coli* from children with various forms of urinary tract infection. *Journal of Pediatrics* **93**, 398–403.

SVANBORG EDÉN, C., GOTSCHLICH, E. C., KORHONEN, T. K., LEFFLER, H. & SCHOOLNIK, G. (1983*a*). Aspects on structure and function of pili on uropathogenic *Escherichia coli*. *Progress in Allergy* **33**, 189–202.

SVANBORG EDÉN, C., HAGBERG, L., HANSON, L. Å., LOMBERG, H., ØRSKOV, I., & ØRSKOV, F. (1982). Bacterial adherence and urinary tract infection. *Lancet* i, 961–962.

SVANBORG EDÉN, C., HAGBERG, L., HANSON, L. Å., HULL, S., HULL, R., JODAL, U., LEFFLER, H., LOMBERG, H. & STRAUBE, E. (1983*b*). Bacterial adherence, a pathogenic mechanism in urinary tract infection caused by *Escherichia coli*. *Progress in Allergy* **33**, 175–188.

SVANBORG EDÉN, C. & HANSSON, H. A. (1978). *Escherichia coli* pili as possible mediators of attachment to human urinary tract epithelial cells. *Infection and Immunity* **21**, 229–237.

SVANBORG EDÉN, C., HANSON, L. Å., JODAL, U., LINDBERG, U. & ÅKERLUND, A. S. (1976). Variable adherence to normal human urinary-tract epithelial cells of *Escherichia coli* strains associated with various forms of urinary-tract infection. *Lancet* ii, 490–492.

SVANBORG EDÉN, C., JODAL, U., KAIJSER, B., LEFFLER, H., LOMBERG, H., ØRSKOV, F. & ØRSKOV, I. (1985). Adhesive properties and O, K, H and F antigens of *E. coli* associated with recurrent pyelonephritis. In *Seminars in Infections Diseases*. Vol. V. *Bacterial Vaccines*. (In the Press.)

TAYLOR, P. W. (1974). Sensitivity of some smooth strains of *E. coli* to the bactericidal action of normal human serum. *Journal of Clinical Pathology* **27**, 626–629.

TAYLOR, P. W. (1975). Genetical studies of serum resistance in *Escherichia coli*. *Journal of General Microbiology* **89**, 57–66.

TAYLOR, P. W. (1976). Immunochemical investigations of lipopolysaccharides and acidic polysaccharides from serum-sensitive and serum-resistant strains of *Escherichia coli* from urinary tract infections. *Journal of Medical Microbiology* **9**, 405–412.

TAYLOR, P. W. (1983). Bactericidāl and bacteriolytic activity of serum against gram-negative bacteria. *Microbiological Reviews* **47**, 46–83.

TAYLOR, P. W. & PARTON, R. (1976). A protein associated with serum resistance in *Escherichia coli*. *Journal of Medical Microbiology* **10**, 225–232.

TAYLOR, P. W. & ROBINSON, M. K. (1980). Determinants that increase the serum resistance of *Escherichia coli*. *Infection and Immunity* **29**, 278–280.

TIMMIS, K. N., MANNING, P. A., ECHARTI, C., TIMMIS, J. K. & MOLL, A. (1981). Serum resistance in *E. coli*. In *Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids* (ed. S. B. Levy, R. C. Clowes and E. L. Koenig), pp. 133–144. New York: Plenum.

TULLUS, K., HÖRLIN, K., SVENSON, S. B. & KÄLLENIUS, G. (1984). Epidemic outbreaks of acute pyelonephritis caused by nosocomal spread of P fimbriated *Escherichia coli* in children. *Journal of Infectious Diseases* **150**, 728–736.

TURCK, M., PETERSDORF, R. G. & FOURNIER, M. R. (1962). The epidemiology of non-enteric *Escherichia coli* infections: prevalence of serologic groups. *Journal of Clinical Investigation* **41**, 1760–1765.

UHLENHUTH, P. (1897). Beiträge zur Pathogenität des *Bacterium coli*. *Zeitschrift für Hygiene und Infektionskrankheiten* **26**, 476–484.

VAHLNE, G. (1945). Serological typing of the colon bacteria. *Acta Pathologica et Microbiologica Scandinavica* **62** (Suppl.), 1–127.

VALVANO, M. A. & CROSA, J. H. (1984). Aerobactin iron transport genes commonly encoded by certain Col V plasmids occur in the chromosome of a human invasive strain of *Escherichia coli* K1. *Infection and Immunity* **46**, 159–167.

VAN DEN BOSCH, J. F., DE GRAAF, J. & MACLAREN, D. M. (1979). Virulence of *Escherichia coli* in experimental hematogenous pyelonephritis in mice. *Infection and Immunity* **25**, 68–74.

VAN DEN BOSCH, J. F., EMÖDY, L., & KÉTYI, I. (1982). Virulence of haemolytic strains of *Escherichia coli* in various animal models. *FEMS Microbiology Letters* **13**, 427–430.

VAN DEN BOSCH, J. F., POSTMA, P., DE GRAAF, J. & MACLAREN, D. M. (1981). Haemolysis by urinary *Escherichia coli* and virulence in mice. *Journal of Medical Microbiology* **14**, 321–331.

VAN DIJK, W. C., VERBRUGH, H. A., PETERS, R., VAN DER TOL, M. E., PETERSON, P. K. & VERHOEF, J. (1979). *Escherichia coli* K antigen in relation to serum induced lysis and phagocytosis. *Journal of Medical Microbiology* **12**, 123–130.

VARIAN, S. A. & COOKE, E. M. (1980). Adhesive properties of *Escherichia coli* from urinary-tract infections. *Journal of Medical Microbiology* **13**, 111–119.

VERWEIJ-VAN VUGHT, A. M. J. J., VAN DEN BOSCH, J. F., NAMAVAR, F., SPARRIUS, M. & MACLAREN, D. M. (1983). K antigens of *Escherichia coli* and virulence in urinary-tract infection. Studies in a mouse model. *Medical Microbiology* **16**, 147–155.

VOSTI, K. G., GOLDBERG, L. M., MONTO, A. S. & RANTZ, L. A. (1964). Host parasite interaction in patients with infections due to *E. coli*. I. The serogrouping of *E. coli* from intestinal and extraintestinal sources. *Journal of Clinical Investigation* **43**, 2377–2385.

VÄISÄNEN-RHEN, V. (1984). Fimbria-like hemagglutinin of *Escherichia coli* O75 strains. *Infection and Immunity* **46**, 401–407.

VÄISÄNEN-RHEN, V., ELO, J., VÄISÄNEN, E., SIITONEN, A., ORSKOV, I., ORSKOV, F., SVENSON, S. B., MÄKELÄ, P. H. & KORHONEN, T. K. (1984). P-fimbriated clones among uropathogenic *Eschericia coli* strains. *Infection and Immunity* **43**, 149–155.

VÄISÄNEN, V., KORHONEN, T. K., JOKINEN, M., GAHMBERG, C. G. & EHNHOLM, C. (1982). Blood group M specific haemagglutinin in pyelonephritogenic *Escherichia coli*. *Lancet* i, 1192.

WAALWIJK, C. & DE GRAAF, J. (1983). Inactivation of haemolysin production in *Escherichia coli* by transposon insertion results in loss of virulence. *Antonie van Leeuwenhoek; Journal of Microbiology and Serology* **49**, 23–30.

WAALWIJK, C., MACLAREN, D. M. & DE GRAAF, J. (1983). In vivo function of hemolysin in the nephropathogenicity of *Escherichia coli*. *Infection and Immunity* **42**, 245–249.

WAALWIJK, C., VAN DEN BOSCH, J. F., MACLAREN, D. M. & DE GRAAF, J. (1982). Hemolysin plasmid coding for the virulence of a nephropathogenic *Escherichia coli* strain. *Infection and Immunity* **35**, 32–37.

WARNER, P. J., WILLIAMS, P. H., BINDEREIF, A. & NEILAND, J. B. (1981). Col V plasmid-specified aerobactin synthesis by invasive strains of *Escherichia coli*. *Infection and Immunity*. **33**, 540–545.

WEBB, L., GOODWIN, C. S. & GREEN, J. (1982). O antigen loss by semirough *E. coli* causing recurrent urinary infections, analysed by gel column filtration and gas–liquid chromatography. *Pathology* **14**, 17–24.

WEINSTEIN, R. J. & YOUNG, L. S. (1976). Neutrophil function in gram-negative rod bacteremia: the interaction between phagocytic cells, infecting organisms and humoral factors. *Journal of Clinical Investigation* **58**, 190–199.

WEINSTEIN, R. & YOUNG, L. S. (1978). Phagocytic resistance of *E. coli* K1 isolates and relationship to virulence. *Journal of Clinical Microbiology* **8**, 748–755.

WELCH, R. A., DELLINGER, E. P., MINSHEW, B. & FALKOW, S. (1981). Haemolysin contributes to virulence of extra-intestinal *E. coli* infections. *Nature* (*London*) **293**, 665–667.

WILLIAMS, P. H. (1979). Novel iron uptake system specified by Col V plasmids: an important component in the virulence of invasive strains of *Escherichia coli*. *Infection and Immunity* **26**, 925–932.