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Virulence Properties of Escherichia coli Strains in Patients With Chronic Pyelonephritis

Summary: In 50 *Escherichia coli* strains obtained from the bladder puncture urine of patients with chronic pyelonephritis, determinations of virulence properties were performed. All of the *E. coli* strains isolated from 26 acute episodes of pyelonephritis were found in the smooth form. 30% possessed K 1 antigen, 77% showed the ability to form hemolysin and 30% produced colicin V (aerobactin). Fimbriae (detected by mannose-resistant hemagglutination) were registered in 81%, and plasmids ranging between 50 and 70 Md were demonstrated in 70% of the bacteria. In contrast to this, only 70% of the *E. coli* strains isolated from 24 patients at an inactive stage of pyelonephritis were found in the smooth form; 10% of these encoded K 1 antigen, 20% hemolysin and 10% colicin V. Plasmids in the range 50 to 70 Md could be found in 30%. On the basis of multivariate analysis of variance and discriminant analysis, it was confirmed that uropathogenic strains possess several virulence properties, mannose-resistant hemagglutination being of particular importance.

Zusammenfassung: *Virulenzeigenschaften bei Escherichia coli-Stämmen von Patienten mit chronischer Pyelonephritis.* Bei 50 *Escherichia coli*-Stämmen, die aus dem Blasenpunktionsurin von Patienten mit chronischer Pyelonephritis gewonnen wurden, erfolgten Bestimmungen ihrer Virulenzeigenschaften. In den 26 Fällen mit einer akuten Erkrankung fanden sich ausschließlich S-Formen der Bakterien, K 1 Antigen wurde in 30% nachgewiesen, 77% verfügten über die Fähigkeit zur Hämolysinbildung und 30% über die zur Colicin V (Aerobactin)-Produktion. In 70% fanden sich Plasmidspezies der Größe zwischen 50 und 70 Md. Demgegenüber wurden bei den 24 Patienten mit einem inaktiven Stadium der Pyelonephritis nur in 70% der *E. coli*-Stämme S-Formen gefunden. Bei ihnen konnten in 10% K 1 Antigen, in 20% Hämolysin und in 10% Colicin V bestimmt werden. Plasmide zwischen 50 und 70 Md. lagen in 30% der Bakterienstämme vor. Nach der multivariaten Varianz- und Diskriminanzanalyse bestätigt sich, daß uropathogene Bakterienstämme über mehrere Virulenzeigenschaften verfügen müssen, dabei kommt der Fähigkeit zur mannoseresistenten Hämagglutination eine besondere Bedeutung zu.

Introduction

It is known from investigations by *Glynn* et al. (1), *Kaijser* (2), *Bjorksten* and *Kaijser* (3), *Minshew* et al. (4) and *van den Bosch* et al. (5) that the ability of *Escherichia coli* strains to produce urinary tract infections correlates with specific properties of the microorganism.

There are several reports concerning the detection of virulence properties of *E. coli* strains pathogenic to the urinary tract, such as their ability to produce hemolysin and colicin as well as mannose-resistant or mannose-sensitive hemagglutination (6–11).

We tried to investigate a complex of virulence factors in *E. coli* strains in patients with chronic pyelonephritis, as well as to evaluate their correlations with the clinical picture and the course of urinary tract infection.

Patients and Methods

Patients

In 50 patients (48 women and two men; mean age 36.9 ± 13.9 years) with chronic pyelonephritis, *E. coli* were isolated from urine specimens taken by suprapubic bladder puncture. All patients were attending our out-patient unit. An *E. coli* bacteriuria had already been identified in earlier consultations. Each patient was followed for more than three years. The diagnosis of chronic pyelonephritis was established by clinical and laboratory findings over a period of more than three years and by radiographic data (focal scarring of the kidneys), caliceal clubbing and blunting. 26 patients presented with an acute episode of the disease with loin pain, increased temperature (> 38.5°C), increased erythrocyte sedimentation rate (> 25 mm/h), leukocytosis (> 8 Gpt/l), increased α_2-globulin fraction, positive C-reactive protein, leukocyturia and bacteriuria.

At the time of the investigation, 24 patients exhibited no clinical or biochemical signs of process activity.

Methods

Microbiological investigations

Bacteria: The bacteriological examination included the bacterial

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Table 1: Virulence factors of *Escherichia coli* strains from patients with chronic pyelonephritis (absolute and relative frequencies).

Virulence factors	Acute exacerbation n =26		Inactive stage n = 24		Odds ratio
	Absolute	Relative	Absolute	Relative	
Smooth form (S-form)	26	1.0	16	0.7	27.3*
K 1 antigen	9	0.3	3	0.1	3.3
Fimbriae (detection by electron microscopy)	23	0.9	11	0.5	7.9*
Fimbriae (mannose-resistant hemagglutination)	21	0.8	∅	∅	191.5*
Hemolysin formation	20	0.8	5	0.2	11.2*
Colicin V production	9	0.3	2	0.1	4.9
Plasmids (50–70 Md)	17	0.7	7	0.3	4.5

* Significant difference between acute exacerbation and inactive stage $p \leq 0.05$.

count, differentiation of the species and resistance testing according to *Edwards* and *Ewing* (12).

S- and R-forms of E. coli: The rough character (R-form) of the strains was identified by demonstrating spontaneous agglutination of the bacterial suspensions in 3.5% saline after heating for 2 h in the steampot (13, 14).

K 1 antigen: The detection of K 1 antigen was carried out by means of K 1 specific phages (14, 15). Confluent or semiconfluent lysis by K 1 phages of the strain examined were regarded as documentation of K 1 antigen.

Fimbriae

Electron microscopic analysis: In order to identify fimbriae by electron microscopy, the negative staining and surface replica procedures were applied (16, 17).

Mannose-sensitive and mannose-resistant hemagglutination: Hemagglutination was tested using a slide agglutination test according to *Evans* (18). Washed red blood cells (group A) from man, cattle and guinea pigs were used in a 5% solution, and a drop of 0.1 M mannose was added at a ratio of 1:4. The strain to be studied was incubated for 24 h on CFA agar according to *Evans*. Single colonies were suspended in a drop of red blood cells with or without adding D-mannose. Mannose-resistant hemagglutination (evidence of F 7 to 11 fimbriae) was recorded in cases where agglutination of group A erythrocytes from man but not from cattle or guinea pigs took place, even in the presence of D-mannose. Mannose-sensitive hemagglutination (evidence of F 1 fimbriae) occurred only in the absence of mannose.

Hemolysin: The hemolytic activity of the bacterial strains was determined as described by *Springer* and *Goebel* (19). The amount of hemoglobin released was identified spectrophotometrically at 420 nm and served as a quantitative measurement of the hemolytic activity of the strains.

Colicin V production: Based on the method of *Smith* and *Huggins* (20), the strains were tested for colicin V production using the double layer procedure. *E. coli* K 12/J 53, both with and without colicin V plasmid, served as indicator. Strains that produced an inhibitory halo in indicator J 53, but not in indicator Col-V-J 53, were regarded as colicin V producers. All colicin V positive strains were demonstrated to be aerobactin producers (21, 22).

Plasmid analysis: Plasmid analysis was performed according to *Tietze* and *Tschäpe* (23). In order to isolate plasmid DNA, the *E. coli* strains were subjected to alkaline lysis. All plasmids were identified by means of agarose gel electrophoresis in Tris borate buffer. To determine the exact size of smaller DNA species, characterization according to *Meyer* (24) was necessary. An es-

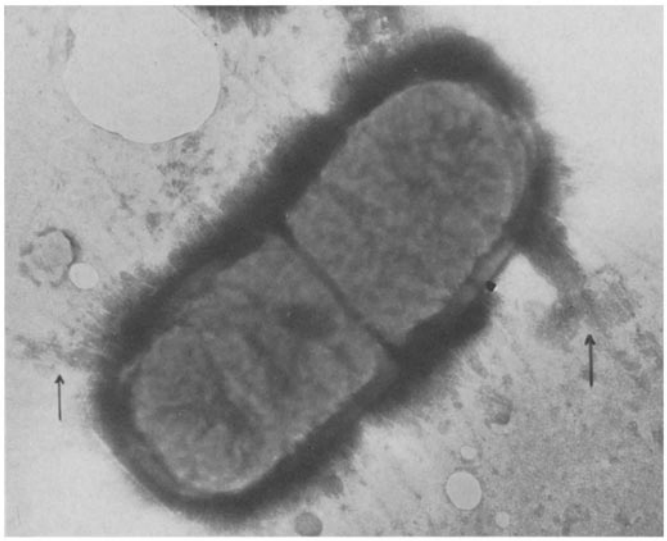

Figure 1: Electron micrograph. *Escherichia coli* O 75: detection of fimbriae by electron microscopy, capacity for mannose-resistant hemagglutination, demonstration of hemolysin formation. 60,000-fold magnification, negative staining.

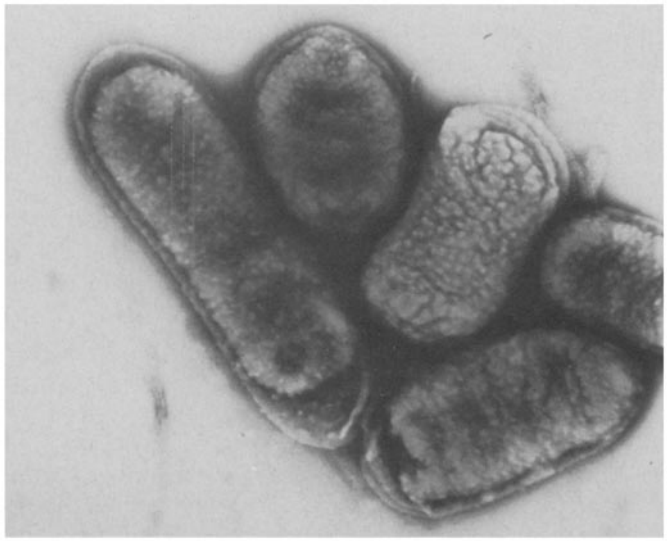

Figure 2: Electron micrograph. *Escherichia coli* R-form, 56,000-fold magnification, negative staining.

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Table 2: Incidence of virulence factors in *Escherichia coli* S- and R-forms (absolute and relative frequencies).

Virulence factors	S-form n = 42		R-form n = 8		Odds ratio
	Absolute	Relative	Absolute	Relative	
K 1 antigen	10	0.2	2	0.3	0.8
Fimbriae (detection by electron microscopy)	30	0.7	4	0.5	2.4
Fimbriae (mannose-resistant hemagglutination)	19	0.5	2	0.3	2.2
Hemolysin formation	22	0.5	3	0.4	1.7
Colicin V production (debated)	10	0.2	1	0.1	1.6

timation of the plasmid sizes (molecular size ranges) was obtained by comparison with reference plasmids (23).

Statistical analysis: The calculation of nominal scaled frequency distribution was carried out by relative risk w (odds ratio), according to the formula of *Ascombe* (25). In order to check the statistical hypothesis $w \neq 1$, the X^2-test with Yates' correction appeared suitable. To examine the possibility of discriminating strains by their virulence properties, the multivariate analysis of variance and the discriminant analysis according to the algorithms of *Ahrens* and *Läuter* (26) were used.

Results

Incidence of Virulence Factors

E. coli strains isolated from patients with acute infection demonstrated virulence factors more often than strains encountered in patients during a symptom-free (inactive) interval.

The frequency distribution of virulence factors in relation to the microorganisms of both groups studied is shown in Table 1. The S-form of bacteria was demonstrated in 42 cases and the R-form in eight.

Microorganisms occurring during an acute episode of pyelonephritis were found exclusively in the smooth form. K 1 antigen was detected in 12 *E. coli* strains, nine (35%) from an active disease and three (13%) from inactive pyelonephritis.

Fimbriae were identified by electron microscopy in 34 strains. They were more frequently detectable in microorganisms producing an acute course of disease (88%) than in those causing an inactive disease (46%). Figures 1 and 2 show electron microscopic photographs of *E. coli* with and without fimbriae. Hemagglutination sensitive to mannose (mannose-sensitive hemagglutination) was registered in 45 strains. 21 *E. coli* strains possessed a capacity for mannose-resistant hemagglutination. Without exception, these organisms occurred during acute infection. In 20 strains, we simultaneously observed mannose-sensitive and mannose-resistant hemagglutination. Hemolysin was formed by half of all bacterial strains. Organisms causing an acute infection possessed this virulence property more frequently (77%) than those isolated during an inactive disease (21%). 11 strains had the ability to produce colicin, nine of which were found in patients suffering from an acute episode of pyelonephritis. Plasmid analysis demonstrated a large number of plasmids between 1.0 and 160 megadaltons. The number of plasmid species between 50 and 75 megadaltons was remarkable; they were found in 17 (65%) strains isolated during an acute disease and in seven (29%) organisms causing asymptomatic bacteriuria.

Combination of Virulence Factors

Only one strain possessed no virulence factors at all. Most frequently, three properties were found in common (n = 14; in one *E. coli* strain, seven factors were even analyzed).

The combination of mannose-resistant hemagglutination and the production of hemolysin was observed 14 times. K 1 antigen and the ability to produce hemolysin were demonstrated in four organisms. K 1 antigen, fimbriae and the ability to produce hemolysin and colicin V were more often detected in the S-forms of *E. coli* than in the R-forms (Table 2). However, statistically significant differences in the occurrence of virulence characteristics between S- and R-forms of *E. coli* were not found.

Analysis of the Virulence Determinants

The development of specific properties of virulence is determined by chromosomal and extrachromosomal genes. Based on the result of plasmid analysis as well as on the contribution of hemolysin formation, mannose-resistant hemagglutination and colicin V production, we have attempted to classify the strains of bacteria into "uropathogenic" and "non-uropathogenic", utilizing the information on the genetic structure (evidence of virulence determinants). According to this classification, an *E. coli* strain was considered "uropathogenic" if at least two virulence factors or plasmid species of 50–75 megadaltons were simultaneously detectable. By these criteria, 38 *E. coli* strains were classified as "uropathogenic" (Figure 3). All organisms appearing in patients with an acute infection corresponded to this classification.

Multivariate Analysis for the Assessment of the Properties Studied in the Escherichia coli Strains

The statistical evaluation of the *E. coli* strains studied included the following findings:

1. Occurrence as S- or R-form;
2. Detection of K 1 antigen;
3. Capacity for mannose-resistant hemagglutination;

4. Hemolysin formation;
5. Colicin V production;
6. Electron microscopic identification of fimbriae;
7. Analysis of the virulence determinants.

Univariate analysis of variance revealed that "mannose-resistant hemagglutination" (3 above) and analysis of the "virulence determinants" (7 above) had the highest separation measure encountered at an active and inactive stage of disease ($\alpha \leqq 0.0001$). This fact is also reflected in the multivariate study of all features of the *E. coli* strains. The optimum set of factors that characterizes the virulence of a strain occurring in the urinary tract consists of these two features ($\alpha \leqq 0.0001$). The result of discriminant analysis is summarized in Table 3.

The results of our study allow us to state the following:

1. 21 of the 26 strains of bacteria causing an acute infection could be classified by the property "mannose-resistant hemagglutination" and by analysis of the virulence determination (categorization of possible uropathogenicity) (7 above).
2. No organisms isolated during a clinically inactive course of pyelonephritis were assigned to the class "active pyelonephritis".

According to these results, strains with the capacity for mannose-resistant hemagglutination as well as appropriate virulence determinants, including certain plasmids, will be able to cause an acute infectious disease with a high degree of probability (80 out of 100 cases).

From the discrimination result "acute pyelonephritic stage", conclusions may be drawn with a high degree of certainty about organisms causing an acute course of disease (a probability of 1 was found using the test mentioned above). Conversely, strains appearing during an inactive course can be recognized as such in all cases.

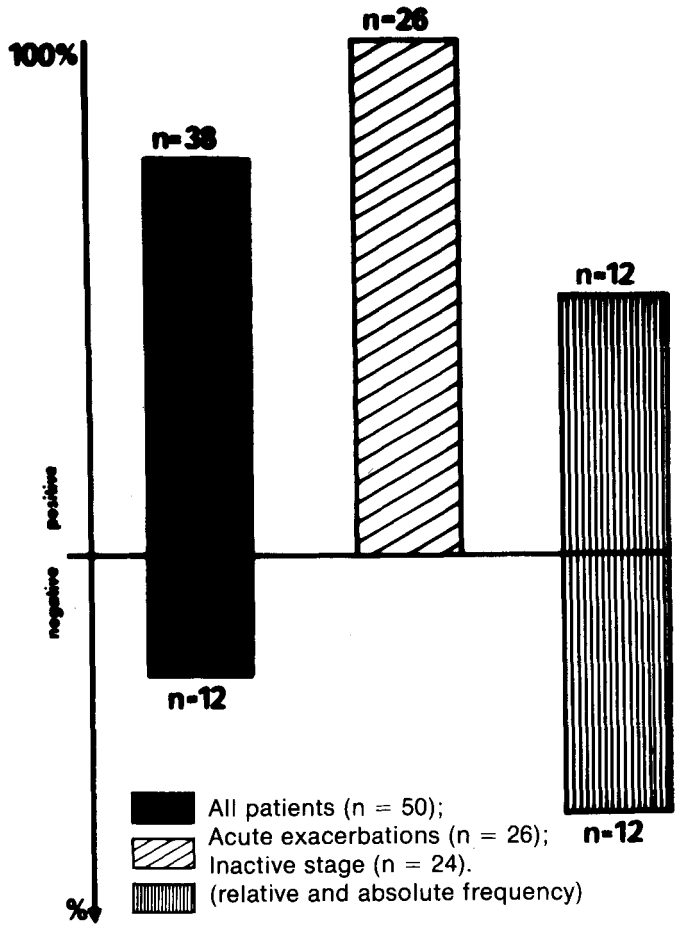

Figure 3: Assessment of "uropathogenicity" by analysis of the virulence determinants of *Escherichia coli* strains in patients with chronic pyelonephritis.

Discussion

The course of an infection is dependent on the ability of the microorganisms to resist the complex defence systems of the human body. Investigations into the properties of uropathogenic strains seem particularly warranted in the case of *E. coli*, since they are responsible for the most frequent infections of the urinary tract and the kidneys (15, 27–29).

In such diseases, there is a predominance of *E. coli* strains that do not permit a serofermentative differentiation from ubiquitous intestinal organisms (9, 30, 31). Indeed, these bacteria are especially remarkable with respect to the phenotype hemolysin formation, mannose-resistant hemagglutination, serum resistance and iron sequestering (4, 8, 9, 32–34). Although the determination of single features of virulence is of interest in characterizing special capacities of a given strain, the classification of an organism as "virulent" or "avirulent" for clinical purposes will only be possible when several properties of virulence are demonstrated at the same time.

In the 50 *E. coli* strains isolated from the urine specimens taken by bladder puncture from patients with a clinically established pyelonephritis, virulence features were observed at a varying frequency. All patients were treated as out-patients for chronic urinary tract infections, the observation period lasting in all cases for at least three years. An *E. coli* bacteriuria had already been identified during previous examinations.

The comparison between the strains from the patient group with an acute episode of pyelonephritis and those from patients at a clinically inactive stage of pyelonephritis appeared to be interesting, because there were differences in the virulence properties of the *E. coli* strains. Organisms causing acute disease courses showed exclusively S-forms; among these, K 1 antigen, fimbriae (electron microscopy) and the properties for hemolysin and colicin V production could more frequently be detected.

The capacity for mannose-resistant hemagglutination was observed only in strains that had induced an acute infection (Table 1). However, *E. coli* strains that were isolated from patients with clinically inactive pyelonephritis also possessed virulence properties. It is remarkable to note that only in this group R-forms of *E. coli* were observed.

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Table 3: Result of multivariate analysis of variance and discriminance analysis for determining the virulence of *Escherichia coli* strains.

Optimal set for characterization of virulence	F_3 Capacity for mannose-resistant hemagglutination F_3 Assessment of virulence determinants ("uropathogenicity")		
Result of discrimination (desubstition)	Result of testing discriminant function (y)		
	Acute exacerbation class 1	Inactive stage class 2	Sum total
Acute exacerbation	21	5	26
Inactive stage	0	24	24
Sum total	21	29	50
Error of discrimination			
Sensitivity (proper division in class 1):		0.8077	
Specificity (proper division in class 2):		1.0	
Predictive value (prevalence of acute cases):		0.52	
pvp (predictive value positive: acute):		1.0	
pvn (predictive value negative: inactive):		0.8276	

Despite the loss of the O-specific polysaccharide, R-forms also have to be regarded as potent organisms causing urinary tract infections, as virulence factors were also found in these *E. coli* strains (Table 2); *Naumann* (35) also registered a homologous immune response to R-forms in the host organism. On the other hand, the increased occurrence of R-forms in relapsing pyelonephritis might be considered as an expression of immune tolerance to S-forms of bacteria.

So far, uropathogenic strains of bacteria can mainly be characterized by the properties mannose-resistant hemagglutination (evidence of fimbriae), hemolysin formation and colicin V production (serum resistance, iron sequestering) (4, 9, 30, 36). Based on these features, we attempted to classify the *E. coli* strains according to their possible "uropathogenicity". It thus appeared that 37 of 50 *E. coli* strains had to be regarded as "uropathogenic"; this represented all the strains from patients with an acute pyelonephritic episode (see Tables 2 and 3). This assessment must be a prospective one, because molecular-genetic investigations do not yet permit an exact characterization of single functions. Analysis of the pathogens of a larger patient population with urinary tract infection and monitoring of the disease course seem to be necessary in order to confirm typical combinations of virulence factors and plasma profiles in strains of bacteria, as well as to give a more detailed characterization of their function. With the aid of the statistical procedures of multivariate analysis of variance and discriminant analysis, the significance of both single features and, in particular, of feature complexes can be assessed (26). Thus, the combined effect of single factors could be judged, with simultaneous consideration of the other properties of the strains involved. As a result of the analyses it was found with a sensitivity of 0.81 and a specificity of 1.00 that *E. coli* strains causing an acute infection must possess an appropriate genetic structure (analysis of virulence determinants) and the capacity for mannose-resistant hemagglutination. Accordingly, a strain possessing no fimbriae that are demonstrable by the determination of mannose-resistant hemagglutination will hardly be able to induce an acute infection. This result of the multidimensional feature assessment of microorganisms is confirmed by the results of investigations by *Lomberg* et al. (37), *Svanborg Edén* et al. (38), *Källenius* (39) and *Korhonen* et al. (40).

Thus, strains possessing the ability to colonize and to cytotoxically damage cells and tissues may possibly initiate acute infections. On the other hand, properties favouring the survival of the strains in the tissues (serum resistance, iron sequestering, K 1 antigen) tend to produce relapse and chronic infection.

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